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Joana Antunes de Lima Bastos

Incidence and prevalence of *Helicobacter pylori* infection in three Portuguese cohorts

Porto | 2013

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Dissertação de candidatura ao grau de Doutor apresentada à Faculdade de Medicina da Universidade do Porto

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ABSTRACT

Incidence and prevalence of *Helicobacter pylori* infection in three Portuguese cohorts

Helicobacter pylori infection is the most important determinant for gastric cancer. The frequency of the infection in adulthood reflects the combined effect of a birth-cohort phenomenon and sustained acquisition throughout life. The occurrence of the different gastric outcomes of *H. pylori* infection also depends on the age at acquisition. Therefore, this thesis aims to identify factors associated with *H. pylori* infection at different ages (distinguishing the potentially critical periods of childhood and adolescence, in addition to adulthood), through the following specific objectives:

1) To quantify the prevalence and the incidence of *H. pylori* infection and the proportion of subjects infected with CagA-positive strains in a cohort of adults (EPIPorto) and to identify the major sociodemographic correlates of the infection (study I).
2) To quantify the prevalence and the incidence of *H. pylori* infection in a cohort of adolescents (EPITeen) and to identify risk factors for the infection (study II).
3) To quantify the prevalence of *H. pylori* infection in a cohort of children evaluated at ages between 4 and 5 years (Geração XXI), and to assess the independent effect of child-care attendance in the early life acquisition of the infection (study III).
4) To quantify the association between child-care attendance and *H. pylori* infection through systematic review and meta-analysis (study IV).

These objectives were achieved through the conduction of four different studies:

Study I

Socio-demographic determinants of prevalence and incidence of *Helicobacter pylori* infection in Portuguese adults

A study based on the EPIPorto cohort (a cohort of non-institutionalised adult dwellers in the city of Porto) was conducted to estimate the prevalence and incidence of *H. pylori* infection and to identify its major socio-demographic correlates in an urban population from North Portugal. Participants were recruited between January 1999 and December 2003 by random digit dialling, using households as the sampling frame, followed by simple random sampling to select one eligible person among permanent residents in each household, without allowing replacement of refusals. The selected participants were invited to visit the Department of Clinical Epidemiology, Predictive Medicine and

Public Health to a face-to-face interview and to perform a physical examination. A venous blood sample was collected after 12-hour overnight fast and was stored at -20ºC until analysis. The EPIPorto cohort included 2485 participants aged 18 to 92 years, corresponding to a participation proportion of 70%. For the present study, *H. pylori* infection status was assessed in 2067 subjects (83%). During a follow-up conducted between 2005 and 2008, 1682 participants were re-evaluated, using a structured questionnaire similar to the one used during the cohort assembling, and information was collected on social, demographic, personal and family medical history, and behavioural characteristics. A physical examination was performed and anthropometric measurements, blood pressure, and a fasting blood sample were available for each participant. *H. pylori* infection status was assessed in 114 (41%) participants without evidence of infection at the baseline. Serum anti-*H. pylori* IgG titres were assessed by ELISA. To quantify the prevalence of infection with CagA-positive strains at baseline, a subsample of 412 participants classified as infected according to the anti-*Helicobacter pylori* IgG titres, selected to be representative of the infected individuals at the baseline regarding sex, age and education, was further tested by Western Blot. Modified Poisson and Poisson regression models were used to estimate crude and sex-, age-, and education-adjusted prevalence ratios (PR) and incidence rate ratios (RR), respectively, and the corresponding 95% confidence intervals (CI). The prevalence of *H. pylori* infection was 84.2% (95%CI: 82.4-86.1). It increased across age groups in the more educated subjects, (18-30 years: 72.6%; ≥71 years: 88.1%; P for trend<0.001) and decreased with education in the younger (≤4 schooling years: 100.0%; ≥10 schooling years: 72.6%; P for trend<0.001). Living in a more deprived neighbourhood was associated with a higher risk of infection, only in the younger (PR=1.20, 95%CI: 1.03-1.38) and more educated participants (PR=1.15, 95%CI: 1.03-1.29). Among the infected, the proportion with CagA-positive strains was 61.7% (95%CI: 56.6-66.9). The incidence rate was 3.6/100 person-years (95%CI: 2.1-6.2), lower among the more educated (≥ 10 *vs.* ≤ 9: RR=0.25, 95%CI: 0.06-0.96). The seroreversion rate was 1.0/100 person-years (95%CI: 0.6-1.7).

Study II

Prevalence, incidence and risk factors for *Helicobacter pylori* infection in a cohort of Portuguese adolescents (EPITeen)

A study based on the EPITeen cohort was conducted to estimate the prevalence of *H. pylori* infection at the age of 13 and the incidence after a 3-year follow-up and to identify risk factors for infection at different ages. The EPITeen cohort is a representative sample of adolescents born in 1990 and studying in Porto. Participants

were recruited in the public and private schools from Porto, in 2003/2004. During the baseline evaluation, 2160 students (1651 from public and 509 from private schools), corresponding to a participation proportion of 78%, were considered eligible for the study and answered two standardized questionnaires, one at home, with the legal guardians, and other at school, during the team visit. The home questionnaire inquired about demographic, social, behavioural and clinical characteristics of the participant and family. The school questionnaire comprised questions regarding physical activity, smoking and alcohol intake. A physical examination was performed at school, between 8 a.m. and 10 a.m., by a team of experienced nurses, nutritionists and physicians, following standardized procedures. A 12-hour overnight intravenous blood sample was obtained from 1390 participants. For the present study, *H. pylori* infection status was assessed for 1312 subjects (94.4%) from which a large enough aliquot was available. During a follow-up conducted between 2007 and 2008, 1716 (79%) participants were re-evaluated following the same procedures used at the baseline evaluation. Information regarding *H. pylori* infection was available for 280 (63%) participants without evidence of infection at the baseline. Serum anti-*H. pylori* IgG titres were assessed by ELISA. Binomial regression was used to estimate parental education-adjusted prevalence ratios (PR) and the corresponding 95% confidence intervals (CI). Poisson regression was used to compute parental education-adjusted incidence rate ratios (RR) and the corresponding 95% CI. The prevalence of *H. pylori* infection was 66.2%, lower in subjects with more educated parents (≥ 12 *vs.* ≤ 4 schooling years: PR=0.72, 95%CI: 0.63-0.82), and higher for those having more than one sibling (> 1 *vs.* ≤ 1 sibling: PR=1.10, 95%CI: 1.02-1.19) and for smokers (ever *vs.* never: PR=1.11, 95%CI: 1.02-1.20). The incidence rate was 4.1/100 person-years (95%CI: 3.0-5.8). Smoking (ever *vs.* never: RR=2.35, 95%CI: 1.16-4.75) and type of school (private *vs.* public: RR=0.38, 95%CI: 0.16-0.95) were associated with the incidence of infection.

Study III

The role of child-care attendance in the early life prevalence of *Helicobacter pylori* infection: results from the Portuguese birth cohort Geração XXI

The prevalence of *H. pylori* infection and the role of child-care attendance in the early life acquisition of the infection were assessed in the participants of the Geração XXI cohort at the ages 4/5 years. This birth cohort was assembled between 2005 and 2006 at all 5 public maternity units covering the metropolitan area of Porto, Portugal. The present analyses included 1047 children, 1036 mothers and 409 fathers for whom the characterization of the *H. pylori* infection status was accomplished. Serum anti-*H. pylori* IgG titres were assessed by ELISA. Information was collected on the different child-

care options since birth, and on the age at beginning and end of care in each setting, when applicable. Child-care could be provided by parents or other family members in their houses, in day-care homes (care provided by a non-family member at her/his house) or day-care centres (care provided in institutions receiving a large number of children). The child-care settings that were being attended by the children at the time of the interview were also characterized regarding the number of hours per week spent in each setting and the number of children that were cared together by the same provider(s). The independent relation between the attendance of group-care since birth and the *H. pylori* infection status of the children was quantified through odds ratios (OR), and the corresponding 95% confidence intervals (CI), computed using non-conditional logistic regression including terms for children's age, number of siblings, parental education and infection status, and attendance of different types of day-care, as applicable. The prevalence of *H. pylori* infection was 30.6% (95CI%: 27.9-33.6), and it increased significantly with the cumulative time of attendance of day-care centres/homes (from 13.2% among never attendees to 40.2% among those attending >36 months, P for trend <0.001). The OR was 4.88 (95%CI: 2.55-9.35) among those attending these institutions for more than three years, in comparison with never attendees.

Study IV

Child-care attendance and *Helicobacter pylori* infection: systematic review and meta-analysis

The association between child-care attendance and *H. pylori* infection in childhood or adolescence was quantified through systematic review and meta-analysis. PubMed® was searched from inception to July 2012, to identify studies addressing the association between child-care attendance and *H. pylori* infection in childhood or adolescence. The DerSimonian and Laird method was used to compute summary odds ratio (OR) estimates and 95% confidence intervals (CI), heterogeneity was quantified with the I^2 statistic and explained through stratified analyses and meta-regression. Sixteen studies compared subjects attending child-care with those not exposed. The summary OR was 1.12 (95%CI: 0.82-1.52, I^2=77.4%). Summary estimates were similar for crude and adjusted estimates, and higher when the infection was evaluated in children aged ≤ 3 years (OR=2.00, 95%CI: 0.94-4.29, I^2=55.0%). Studies based on the detection of stool antigens yielded higher estimates (OR=2.65, 95%CI: 1.24-5.66, I^2=36.4%), as well as those conducted in settings with high prevalence of *H. pylori* infection (OR=1.44, 95%CI: 0.94-2.20, I^2=74.3%). In multivariate meta-regression there

was no significant association with any of these variables; taking them into account contributed to reduce the I^2 to 67%.

The main conclusions of this work are:

- In adults, the prevalence of *H. pylori* infection in Portugal remains among the highest in Europe, particularly among less educated individuals; taking this into account, stomach cancer incidence and mortality rates in Portugal are likely to remain among the highest in the World during the next decades.
- *H. pylori* infection is a frequent and early event in Portugal. We identified smoking as a modifiable risk factor for infection during adolescence, and showed that the risk of infection in early childhood is increased by day-care attendance; these are potential targets for prevention of *H. pylori* infection in early life.

RESUMO

Incidência e prevalência da infeção por *Helicobacter pylori* em três coortes Portuguesas

A infeção por *Helicobacter pylori* é um importante determinante para a ocorrência de cancro gástrico. A infeção por *H. pylori* na fase adulta resulta da combinação entre um efeito de coorte e a aquisição durante a vida. A idade em que a infeção é adquirida é um importante determinante do desenvolvimento de patologias gástricas. Assim, esta tese de doutoramento pretende identificar fatores que contribuam para a aquisição da infeção por *H. pylori*, ao longo da vida (distinguindo os períodos críticos da infância e adolescência, além da idade adulta), respondendo aos seguintes objetivos específicos:

1. Quantificar a prevalência e a incidência da infeção por *H. pylori* e a proporção de indivíduos infetados com estirpes CagA-positivas, numa coorte de adultos (EPIPorto) e identificar os principais determinantes sociodemográficos da infeção (estudo I).
2. Quantificar a prevalência e a incidência da infeção por *H. pylori* numa coorte de adolescentes (EPITeen) e identificar os fatores de risco para a infeção (estudo II).
3. Quantificar a prevalência da infeção por *H. pylori* numa coorte de crianças avaliadas entre os 4 e os 5 anos de idade (Geração XXI) e avaliar o efeito independente da frequência de instituições de cuidados infantis na aquisição precoce da infeção (estudo III).
4. Rever sistematicamente a literatura com o objetivo de quantificar a associação entre a infeção por *H. pylori* e o tipo de cuidados que são prestados às crianças (estudo IV).

Para responder a estes objetivos específicos foram desenvolvidos os quatro estudos abaixo descritos.

Estudo I

Socio-demographic determinants of prevalence and incidence of *Helicobacter pylori* infection in Portuguese adults

Foi realizado um estudo de coorte com o objetivo de quantificar a prevalência e a incidência de infeção por *H. pylori* e os seus principais determinantes, numa população adulta do Porto recorrendo à coorte EPIPorto. O recrutamento ocorreu entre janeiro de 1999 e dezembro de 2003 e a seleção dos indivíduos foi feita recorrendo à aleatorização de números de telefone, utilizando a habitação como unidade de

amostragem. Dentro de cada habitação, o participante foi selecionado recorrendo também, a um processo aleatório, não tendo sido permitida a substituição das recusas. Os participantes selecionados foram convidados a visitar o Departamento de Epidemiologia Clínica, Medicina Preditiva e Saúde Pública da Faculdade de Medicina da Universidade do Porto para responder a um questionário estruturado, efetuado por inquiridores treinados e proceder a uma avaliação física. Recolheu-se uma amostra de sangue, após jejum de 12h durante a noite, que foi armazenada a -20ºC até análise. A coorte EPIPorto é constituída por 2485 participantes, com idades compreendidas entre os 18 e os 92 anos, o que corresponde a uma proporção de participação de 70%. Para o presente estudo, obteve-se informação relativa a 2067 participantes (83%). Entre 2005 e 2008, foram reavaliados 1682 participantes, usando um questionário estruturado, semelhante ao aplicado na constituição da coorte. Recolheu-se informação relativa às caraterísticas sociais, demográficas, comportamentais, e à história clínica do participante e da família. Efetuou-se, igualmente, uma avaliação física, fizeram-se medições antropométricas, mediu-se a pressão arterial e recolheu-se uma amostra de sangue. Para este estudo em concreto, obteve-se informação relativa à infeção por *H. pylori* para 114 (41%) indivíduos, que não estavam infetados no momento da constituição da coorte. A avaliação da infeção por *H. pylori* realizou-se através da determinação dos anticorpos anti-*H. pylori* IgG, por ELISA. De modo a quantificar a prevalência de infeção por estirpes CagA-positivas na primeira avaliação da coorte, foi avaliada, por Western Blot, uma subamostra de 412 participantes classificados como infetados por ELISA, selecionada de forma a ser representativa dos indivíduos infetados em relação ao sexo, idade e educação. Utilizou-se regressão de Poisson com estimativas do erro padrão robustas, para determinar as razões de prevalência (RP), ajustadas para o sexo, idade e a escolaridade e os respetivos intervalos de confiança (IC) a 95%. Utilizou-se regressão de Poisson para determinar as razões de incidência (RR) e os respetivos IC 95%. Os resultados obtidos mostram uma prevalência global de 84,2% (IC95%: 82,4%-86,1%), aumentando com a idade nos indivíduos mais escolarizados (18-30 anos: 72,6%; ≥71 anos: 88,1%; P para a tendência <0.001) e diminui com a educação entre os indivíduos mais jovens (≤4 anos de escolaridade: 100,0%; ≥10 anos de escolaridade: 72,6%; P para a tendência <0.001). Os indivíduos que habitam em áreas mais pobres apresentam um maior risco de infeção, mas apenas nos indivíduos mais jovens (RP=1,20, IC95%: 1,03-1,38) e mais escolarizados (RP=1,15, IC95%: 1,03-1,29). Nos indivíduos infetados por ELISA na primeira avaliação, 61,7% (IC95%: 56,6%-66,9%) eram seropositivos para CagA. A taxa de incidência foi 3,6/100 pessoas-ano (IC95%: 2,1-6,2), menor nos indivíduos

mais escolarizados (≥10 *vs.* ≤ 9: RR=0,25, IC95%: 0,06-0,96). A taxa de seroreversão foi 1,0/100 pessoas-ano (IC95%: 0,6-1,7).

Estudo II

Prevalence, incidence and risk factors for *Helicobacter pylori* infection in a cohort of Portuguese adolescents (EPITeen)

Realizou-se um estudo de coorte, recorrendo à coorte de adolescentes EPITeen, com o objetivo de estimar a prevalência de infeção por *H. pylori* aos 13 anos de idade, estimar a incidência após 3 anos de seguimento e identificar os fatores de risco para a infeção em diferentes idades. A coorte EPITeen é uma amostra representativa de adolescentes nascidos em 1990 e que estudavam na cidade do Porto, em 2003/2004. Durante a constituição da coorte, 2160 adolescentes (1651 estudantes em escolas públicas e 509 estudantes em escolas privadas), o que corresponde a uma proporção de participação de 78%, foram considerados elegíveis para esta amostra e responderam a dois questionários estruturados, um em casa, com a ajuda dos representantes legais, e outro na escola, no momento da visita da equipa de investigação. O questionário de casa dizia respeito às caraterísticas demográficas, sociais, comportamentais e à história clínica do adolescente e da família. O questionário da escola dizia respeito à atividade física e ao consumo de tabaco e álcool. Os participantes realizaram uma avaliação física, efetuada por uma experiente equipa de enfermeiros, nutricionistas e médicos, entre as 8.00h e as 10:00h, seguindo procedimentos predefinidos. Recolheu-se uma amostra de sangue após jejum de 12h durante a noite, em 1390 adolescentes. Para este estudo, avaliou-se a infeção por *H. pylori* em 1312 (94,4%) adolescentes, para quem se dispunha de uma alíquota suficiente. Em 2007/2008, foram reavaliados 1716 (79%) adolescentes seguindo os mesmos procedimentos que os utilizados na constituição da coorte. Para este estudo específico, obteve-se informação relativa à infeção por *H. pylori* para 280 (63%) indivíduos, que não estavam infetados no momento da constituição da coorte. A infeção por *H. pylori* foi avaliada através da determinação dos anticorpos anti-*H. pylori* IgG por ELISA. Foi utilizada regressão Binomial para determinar as razões de prevalência (RP) ajustadas para a escolaridade dos pais e os respetivos intervalos de confiança a 95% (IC). Foi utilizada regressão de Poisson para determinar as razões de incidência (RR) e os respetivos IC 95%. Obteve-se uma prevalência de 66,2%, menor em indivíduos com pais mais escolarizados (≥ 12 *vs.* ≤ 4 anos de escolaridade: RP=0,72; IC95%: 0,63-0,82), e mais frequente para aqueles com mais do que um irmão (> 1 *vs.* ≤ 1 irmão: RP=1,10; IC95%: 1,02-1,19) e para aqueles que afirmaram já terem alguma vez consumido tabaco (alguma vez *vs.* nunca: RP=1,11; IC95%: 1,02-

1,20). A taxa de incidência foi de 4,1/100 pessoas-ano (IC95%: 3,0-5,8). O risco de infeção estava associado ao consumo tabágico (alguma vez *vs.* nunca: RR=2,35; IC95%: 1,16-4,75) e ao tipo de escola frequentado (privada *vs.* pública: RR=0,38, IC95%: 0,16-0,95).

Estudo III

The role of child-care attendance in the early life prevalence of *Helicobacter pylori* infection: results from the Portuguese birth cohort Geração XXI

A prevalência de infeção por *H. pylori* e a relação entre a aquisição de infeção e o tipo de cuidados que são prestados às crianças avaliaram-se em crianças com 4/5 anos de idade, recorrendo à coorte de nascimento Geração XXI. A coorte de nascimento Geração XXI foi recrutada entre 2005 e 2006, nos 5 hospitais públicos com maternidade na área metropolitana do Porto, Portugal. Este estudo em particular inclui 1047 crianças, cujos ambos os pais forneceram uma amostra de sangue no momento da constituição da coorte, 1036 mães e 409 pais. A avaliação da infeção por *H. pylori* realizou-se através da determinação dos anticorpos anti-*H. pylori* IgG por ELISA. No sentido de se caraterizar o tipo de cuidados que foram prestados às crianças, recolheu-se informação desde o nascimento e tendo em conta os momentos em que houve alteração dessa prestação. A informação foi dividida nos seguintes grupos: os cuidados foram prestados em casa pelos pais ou outro familiar; os cuidados foram prestados por alguém externo à família e fora da residência da criança ou os cuidados foram prestados por alguma instituição prestadora de cuidados infantis. A frequência atual de alguma instituição prestadora de cuidados infantis também foi considerada, tendo em conta a duração dessa frequência e o número de crianças que são cuidadas pelo mesmo prestador. Foi utilizada regressão logística não-condicional para determinar os *odds ratios* (OR) e os respetivos intervalos de confiança (IC) a 95%, ajustados para a idade da criança, número de irmãos e educação dos pais, e a frequência de diferentes tipos de prestadores de cuidados infantis, quando aplicável. Os resultados obtidos mostram uma prevalência global de 30,6% (IC95%: 27,9%-33,6%), aumentando com o tempo cumulativo de frequência de instituições de cuidados infantis (de 13,2%, entre aqueles que não frequentaram qualquer tipo de prestador de cuidados infantis, a 40,2%, entre aqueles que frequentaram algum tipo de prestador de cuidados infantis mais de 36 meses; P para a tendência <0,001). O OR era de 4,88 (IC95%: 2,55-9,35) para aqueles que frequentaram esses prestadores mais de 36 meses, quando comparando com aqueles que nunca frequentaram.

Estudo IV

Child-care attendance and *Helicobacter pylori* infection: systematic review and meta-analysis

Reviu-se, sistematicamente, toda a literatura que avaliasse a associação entre a infeção por *H. pylori* e o tipo de cuidados que foram prestados às crianças/adolescentes, publicada na Pubmed®, desde a sua criação até julho de 2012. Utilizou-se o método DerSimonian e Laird para calcular estimativas sumárias de *odds ratio* (OR) e os respetivos intervalos de confiança (IC) a 95%. A heterogeneidade foi quantificada utilizando a estatística I^2 e explicada através de análises estratificadas e utilizando meta-regressão. O OR global foi 1,12 (IC95%: 0,82-1,52, I^2=77,4%). As estimativas conjuntas, quando se consideravam dados brutos, eram semelhantes às estimativas conjuntas, quando se consideravam dados ajustados e maiores quando se consideravam crianças com 3 ou menos anos de idade (OR=2,00, IC95%: 0,94-4,29, I^2=55,0%). Estudos, cujo método de diagnóstico da infeção se baseava na deteção de antigénios nas fezes, apresentavam estimativas sumárias de OR superiores (OR=2,65, IC95%: 1,24-5,66, I^2=36,4%), assim como estudos realizados em regiões com elevada prevalência de infeção por *H. pylori* (OR=1,44, IC95%: 0,94-2,20, I^2=74,3%). No modelo de meta-regressão multivariado não se verificou qualquer associação significativa, com qualquer uma das variáveis acima referidas. A inclusão dessas variáveis no modelo, levou à redução do I^2 para 67%.

As conclusões principais deste trabalho são:

- Nos adultos a prevalência de infeção é muito elevada, permanecendo entre as mais elevadas da Europa, especialmente entre os indivíduos menos escolarizados. Assim sendo, é provável que a incidência e a mortalidade, por cancro de estômago em Portugal, permaneçam entre as mais elevadas do Mundo, nas próximas décadas.
- A infeção por *H. pylori* é um evento frequente e adquirido em idades muito precoces. Verificou-se que o consumo tabágico é um fator de risco modificável para a aquisição da infeção na adolescência e que a frequência de instituições prestadoras de cuidados infantis aumenta o risco de infeção na infância, identificando assim possíveis alvos para a implementação de medidas preventivas

BACKGROUND

HELICOBACTER PYLORI

Helicobacter pylori is a Gram-negative bacterium measuring 2 to 4 μm in length and 0.5 to 1 μm in width (1). These bacteria normally exist as spiral forms that are usually culturable. However, they can convert into viable but nonculturable coccoid forms under stress or they may die as degenerative coccoid forms (2). The species of the genus *Helicobacter* can be grouped in two major lineages, one colonizing the stomach (the gastric) and the other not colonizing the stomach (enterohepatic or nongastric). Both groups are highly organ specific, so that gastric *Helicobacter*, in general, does not colonize the intestine or the liver, and vice versa (1). *H. pylori* belongs to the gastric *Helicobacter* species. These species have adapted to the hostile conditions found at the gastric mucosa surface, and it is currently assumed that the stomachs of all mammals can be colonized by members of the genus *Helicobacter* (1). After entering in the stomach, *H. pylori* produces urease which converts urea into ammonia ions, allowing the survival in this acid environment (3); the chemo attraction of substances such as urea and bicarbonate promote motility toward the mucus layer, where the more pH-neutral conditions allow growth of this species (3). The penetration into the viscous mucus layer is facilitated by the spiral morphology and flagella motility (3).

Despite the high ability to colonize the human stomach, *H. pylori* was identified only in 1982 by Warren and Marshall (4), who were able to show the presence of the bacteria in the stomach of patients with chronic gastritis and gastric ulcers. At the time, the medical community was convinced that the human stomach was sterile. The general opinion was that the extreme acidity of the environment enabled any bacteria to survive for a long period of time. Moreover, they were able to fulfill Koch's postulates for the role of *H. pylori* in antral gastritis, by culturing the bacteria outside the human stomach (4) and by the self-administration of *H. pylori*, by Marshall, a healthy individual with an histologically normal gastric mucosa (5). Marshall was also able to show that infection could be cured by the administration of antibiotics and bismuth salts (6). In 2005, the work of these Australian physicians was worldwide recognized with the award of the Nobel Prize in Physiology or Medicine (7).

However, the history of *H. pylori* infection in humans did not begin in 1982. Indeed, German scientists found spiral-shaped bacteria in the lining of the human stomach in 1875, but they were unable to culture it (8). In 1886, the Polish Professor of Medicine, Jaworski, investigating sediments of gastric washings obtained from humans; found bacteria with a spiral shape, which he called *Vibrio rugula* (9). He was

the first suggesting the possible role of this organism in the pathogenesis of gastric diseases (9). This work was included in the *Handbook of Gastric Diseases*, but, as it was written in Polish, had little impact (9). This discovery was confirmed by Kreinitz in 1906 and after by Doenges, Freedberg and Baron, around 1940 (9). Doenges was able to describe spirochetes in about 40% of human stomachs in *post-mortem* examination and Freedberg and Baron in fresh surgical specimens (9). The latter work was extremely important because indicated that the organisms were gastric pathogens and not merely the result of *post-mortem* contamination (9). Twenty years later, Ito, from the Harvard Medical School, published a photograph showing the bacteria within a parietal cell gland (10). In the 1970s, these spiral bacteria were the subject of a paper by Steer *et al.;* the authors noted that 80% of their gastric ulcer specimens were colonized with these spiral bacteria. However, they were again unable to culture the organism (10).

Nevertheless, it was in animals that the bacterium was firstly well described. In 1893, in Turin, the Italian pathologist Giulio Bizzozero described the presence of spiral bacteria in the stomachs of dogs (11). In 1896, Salomon extended Bizzozero's work and was able to spread these bacteria in mouse stomachs after feeding his mouse colony with gastric scrapings from dogs (11).

Although written reports describing the presence of this bacterium dated only from, approximately, 100 years ago; actually there is anthropologic evidence that this bacterium colonizes humans for at least 3,300 years. Indeed, in 1999, in a study on mummified human remains from the Andean area of South America, Allison *et al.* found that faecal specimens harboured antigens from *H. pylori* nearly 3,000 years old (12). More recently, in 2008, Castillo-Rojas *et al.* found evidence of *H. pylori* infection in Mexican pre-Columbian mummies dating from approximately 1,350 AC (13).

DIAGNOSIS OF *H. PYLORI* INFECTION

The methods to detect *H. pylori* infection can be classified as invasive or non-invasive and are summarized in table 1.

INVASIVE METHODS

Using invasive methods, *H. pylori* infection may be detected by culture, histology or by rapid urease tests using gastric biopsy specimens, which are normally obtained through endoscopy (14). Currently, a positive culture obtained from endoscopy and usually complemented with a biopsy urease test and/or histology is often used as a "gold standard" to detect patients with active *H. pylori* infection (15). Achievement of *H. pylori* infection thorough culture is 100% specific, because after the

growth of the bacteria in culture medium all phenotypic and genotypic identification tests can be made (14). However, the sensitivity is low, as only a relatively portion of the stomach is evaluated, and the distribution of the infection may be irregular (14). In order to avoid false-negative results, the currently recommendation is to take two biopsy specimens from the antrum, one from the anterior and other from the posterior corpus (14). Moreover, culture is extremely dependent of exogenous factors as desiccation, contact with air, temperature and transport (14).

H. pylori infection may also be assessed by histology. As for culture, biopsy specimens are obtained by endoscopy and immediately introduced into a fixative. After storage, the histological slides are prepared and the accuracy of this technique will dependent on the quality of this preparation and of the stain chose (14). Infection is achieved by the observation of the slides and is strongly dependent of the experience of the pathologist and of the time dispended in this diagnosis (14). The sensitivity of this procedure may reach 95%, in optimal conditions, and the specificity is also around 95%, as the presence of other bacteria on the mucosa may lead to false-positive results (14).

H. pylori infection may also be assessed using rapid urease tests. This type of tests has been extensively used since are not expensive, simple to use and transport and give a rapid result (14). The rationale behind this method is that the bacteria, to survival in the acid environment of the stomach, reduces the acidity by the secretion of urease, which catalyses the hydrolyses of the urea, present in the gastric juice, into ammonia and carbon dioxide. This method consists in introducing a biopsy specimen into a urea-rich medium and to have a pH indicator. If there is infection, the urease, produced by the bacteria, will split the urea into ammonia and carbon dioxide and an alkaline environment will be produced; therefore, the pH indicator will change colour (14). The commercial rapid urease tests available have specificities above 95%, but the sensitivity is between 85% and 95%, as it depend on the amount of bacteria need to detect infection and is only adequate to assess active infection (16).

The infection with *H. pylori* may be identified by polymerase chain reaction (PCR), which is a molecular test consisting in DNA amplification. When applicable to the diagnosis of *H. pylori* infection allows its quantification, the detection of specific genes and specific mutations associated with antimicrobial resistance (14). PCR yields information on the presence of potential virulence markers in the strain, allowing the detection of the cag PAI (14). Different primers have been used and some have been developed into commercial kits (16). The sensitivity and the specificity of these methods depends on the assay used, nevertheless they are above 95% (14, 17). This

technique is not widely used as is expensive when compared with culture, histology and rapid urease tests and requires special laboratory conditions.

NON-INVASIVE METHODS

H. pylori infection may also be assessed through non-invasive methods. The bacterium induces a local mucosal and a systemic antibody response showing a transient rise in specific IgM antibodies, followed by a rise in IgG and IgA antibodies that persist during, and after, infection (18). Any of the circulating immunoglobulin isotypes to *H pylori* can be detected by enzyme linked immunosorbent assay (ELISA) antibody or latex agglutination tests (19), however, as IgM antibodies against *H. pylori* are detected only transiently, they have little value for the serological diagnosis of infection (18). The titres of IgA antibodies are high in the majority of infected cases but not in all (14). Therefore, diagnostic commercial and in-house tests have been developed for detection of *H. pylori*-specific IgG antibodies in serum, saliva and urine (14). The accuracy of these methods depends on the method chose and on the commercial test used. Although, detection of *H. pylori*-specific IgG antibodies in saliva in adults has shown a sensitivity of 82% and a specificity of 73% (14); in children, the sensitivity ranges between 64% and 80% and specificity between 86% and 99% (20). In urine, *H. pylori*-specific IgG, in adults, has shown higher sensitivity (93%) and specificity (90%) (14). In children, the sensitivity ranges from 59% to 94% and the specificity from 76% and 97% (20).

Nevertheless, most IgG diagnostic tests are serum-based. These tests are generally simple, reproducible, inexpensive, and can be done on stored samples. They have been used widely in epidemiological studies (15). However, since no single antigen is recognized by all subjects, antigen reagent preparations should contain multiple strains of *H. pylori* (21). Therefore, the accuracy of serological tests depends on the antigens used in the test (15). Nevertheless the sensitivity is high, ranging from 90% to 97%, the specificity is not so high, and homogeneous, ranging from 50% to 96%, in adults (16); in children the sensitivity ranges from 58% to 96% and the specificity from 70% to 99% (20). One of the reasons for the existence of false positives is the gradual decrease in the titres of antibodies after eradication of the infection (14), therefore, the tests, based on antibodies detection, are also called passive detection methods (16), as do not measure actual infection but the exposure to the bacteria at some time.

The diagnosis of the infection is essential, however, it is also extremely important to diagnose the type of infecting bacteria (22), in order to identify more pathogenic strains. The serological techniques available to diagnose *H. pylori* infection

and to determine the cytotoxic type of infecting strains are Western Blotting and ELISA. In Western Blot, the specific antigens are separated by gel electrophoresis, transferred to a filter paper strip and reacted with the patient's serum sample (16). The sensitivity of these tests are similar to ELISA's, although, the identification of the specific antigens make it more specific (14).

The current infection with *H. pylori* infection may also be assessed using non-invasive methods. Non-invasive detection of *H. pylori* by the ^{13}C-urea breath test is based on the assumption that a solution of urea labelled with carbon-13 will be rapidly hydrolysed by the urease enzyme of *H. pylori*. Therefore the patient will ingest urea labelled with either ^{13}C or ^{14}C. The resulting CO_2 is absorbed across the gastric mucosa and then expelled as $^{13}CO_2$, or $^{14}CO_2$, in the expired breath (19). The ^{13}C-urea breath test detects current infection and is not radioactive. It can be used as a screening test for *H. pylori*, to assess eradication and to detect infection in children (15). Both sensitivity and specificity are high (ranging from 90% to 100%), however the test might not be reliable when assessing patients who have had gastric surgery or with the administration of urease inhibiting drugs (23).

Lastly, the infection with *H. pylori* may also be diagnosed using stool tests. These tests can detect either the bacteria or part of it (DNA) and therefore the infection may be assessed by culture, PCR or by the detections of antigens (14). Culture of bacteria in the faeces and the identification through PCR are complex processes (14); therefore, the most usual strategy is to assess infection through the detection of antigens. *H. pylori* antigens may also be detected in faeces using a simple sandwich ELISA. The most widely used tests are those using polyclonal anti-*H. pylori* antibodies coated on microwells. More recently, tests using monoclonal antibodies instead of polyclonal antibodies were developed (14). A meta-analysis, published in 2006 reported global sensitivity of 94% and specificity of 97% (24), with higher accuracy for those using monoclonal antibodies. The main advantage of the test, however, is in epidemiological studies of acquisition of *H. pylori* in children (19).

Table 1. Usual methods for the diagnosis of *H. pylori* infection.

Diagnostic Method	Sensitivity	Specificity	Comments
Invasive methods			
Culture (14)	≈ 95%	100%	Extremely dependent of external factors
Histology (14)	≈ 95%	≈ 95%	Very dependent of the experience of the pathologist and the presence of other bacteria may lead to false positives
Rapid urease test	≈ 90%	≈ 95%	Only detects current infection
PCR (14, 17)	≈ 95%	100%	Allows genotyping. Requires special laboratory conditions
Non-invasive methods			
Antibodies detection (ELISA, Western Blot)			
Urine (14, 20)	≈ 85%	≈ 88%	The test must be done in fresh samples
Saliva (14, 20)	≈ 77%	≈ 83%	Very easy to perform
Serum (16, 20)	≈ 85%	≈ 79%	Detects the exposure to the bacteria, not only current infection
Urea breath tests (23)	≈ 95%	≈ 95%	Only detects current infection
Stool antigens tests (24)	≈ 94%	≈ 97%	Largely used in children

OUTCOMES OF *H. PYLORI* INFECTION

Gastric colonization with *H. pylori* induces histologic gastritis in all infected individuals; however, the clinical consequences depend on the pattern and distribution of gastritis (25). Patients with antral-predominant gastritis, the most common form of *H. pylori* gastritis, are more likely to develop duodenal ulcers, patients with nonatrophic pangastritis are more likely to develop MALT lymphoma and patients with corpus predominant gastritis are more like to have gastric ulcers, gastric atrophy, intestinal metaplasia, dysplasia and ultimately gastric carcinoma (26). Among the infected individuals it is estimated that 10 to 20% will develop ulcer disease, 1 to 2% will develop gastric adenocarcinoma, and less than 0.1%, may be expected to develop mucosa-associated lymphoid tissue (MALT) lymphoma (1). At early stages, the eradication of *H. pylori* can completely cure gastric MALT lymphoma (1). Nevertheless, the majority of the infected individuals will remain asymptomatic.

In 1994, *H. pylori* was classified as carcinogenic to humans by an expert working group from the IARC (27), based on epidemiological evidence for its association with gastric cancer and gastric MALT lymphoma. Since then, evidence has continued to accumulate for the causal role of *H. pylori* in gastric cancer. The strongest epidemiological evidence for the role of *H. pylori* in gastric cancer comes from a combined analysis of 10 prospective studies (28). In this pooled analysis, *H. pylori* was not associated with gastric cardia cancer (RR=1.0, 95%CI: 0.7–1.4), but, for non-cardia

gastric cancers, a 3-fold increased risk was found for infected individuals (RR=3.0, 95% CI: 2.3–3.8). After that, several studies have been published and summarized in systematic reviews and meta-analyses. A very recent meta-analysis (29) shows that when stratifying the data according to the gastric cancer incidence in each population, positive associations were observed in high-risk settings for both cardia (RR=1.98, 95% CI 1.38–2.83) and non-cardia (RR=3.02, 95% CI 1.92–4.74) cancers. These aggregate measures of association may conceal considerable variation between *H. pylori* strains. *H. pylori* is genetically highly diverse, and there is evidence that distinct genetic lineages of *H. pylori* differ in their pathogenicity (1). The most commonly studied pathogenicity genes are the cytotoxin-associated (cagA) gene and the vacuolating cytotoxin (vacA) gene. The vacA gene encodes a vacuolating cytotoxin that is excreted by *H. pylori* and damages epithelial cells, is present in approximately 50% of all strains (1) and shows variation in *H. pylori* strains isolated from different populations worldwide (30). The cagA gene, which is not present in all strains, is considered to be a marker of a pathogenicity island of approximately 35 000 base pairs, encoding a type IV secretion system that transfers the CagA protein into the host cells (31). Infection with cagA-positive strains increases the risk of atrophic gastritis and gastric cancer (32, 33). These findings are in accordance to the findings of the recent meta-analysis, mentioned before, measuring the association between *H. pylori* infection and cardia gastric cancer (29). In this meta-analysis the authors showed a 4-fold increased risk for non-cardia cancer in *H. pylori* Cag A infected individuals. Besides, when stratifying the data according to the gastric cancer incidence in each population, this association only remains in low gastric cancer risk settings (RR = 1.98, 95% CI 1.38–2.83).

Moreover, age at acquisition of infection has been postulated as an important determinant of the *H. pylori*-related gastric outcomes. This was first suggested by Graham *et al.* (34, 35) based on the effect of age of exposition to polio and the occurrence of paralytic poliomyelitis. In 1995, Blaser *et al.* (36), found a positive association, among *H. pylori* infected individuals, between birth order and gastric cancer incidence. The authors follow the assumption that higher-order birth children were earlier exposed at *H. pylori*, or other infectious agents, by their older brothers. It is currently assumed the association between *H. pylori* infection and chronic superficial gastritis (15), with progression to chronic active gastritis, after 30 or more years of a continuous inflammatory response, which may lead to atrophy, decreased gastric acid secretion and, ultimately, to gastric carcinoma (37). But, these results may also be the effect of early exposures, in a period of the life of great vulnerability. Moreover, the birth-cohort pattern observed for gastric and duodenal ulcer could be explained by a

receding *H. pylori* infection accompanied by a simultaneous shift to acquisition at older ages (38), explaining the decreasing rates of gastric carcinoma and the emergence of other diseases, such as duodenal ulcer. Therefore, it is reasonable to assume that the trends of *H. pylori* infection have been responsible for the birth cohort pattern in cancer. Moreover, it has been suggested that high prevalence of infection at younger ages may be associated with higher gastric cancer mortality at an area level, regardless of similarly high frequency of infection in older subjects (39). All this evidence supports the importance of age of acquisition in *H. pylori*-related gastric outcomes and suggests that a better knowledge of *H. pylori* infection at different ages will allow a better understanding of the dynamics of these diseases. Despite the importance of all *H. pylori*-related gastric outcomes, our focus will be in gastric cancer since our studies were developed in a gastric cancer high-risk setting.

GASTRIC CANCER

Gastric cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer death worldwide (40). The distribution of gastric cancer incidence and mortality rates are not homogeneous across geographical areas (40). The high-risk areas are in Japan, China, certain countries in Latin America, Eastern Europe and in Portugal (figure 1 and 2).

Figure 1: Stomach cancer age-adjusted incidence rates in 2008 per 100,000

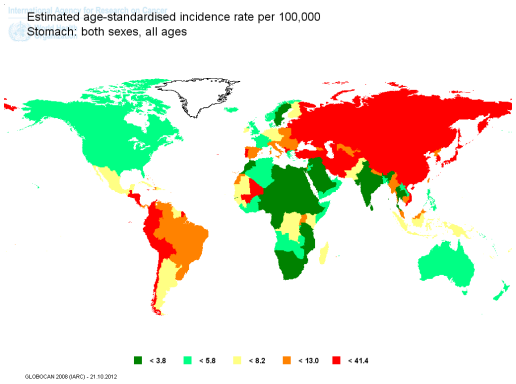
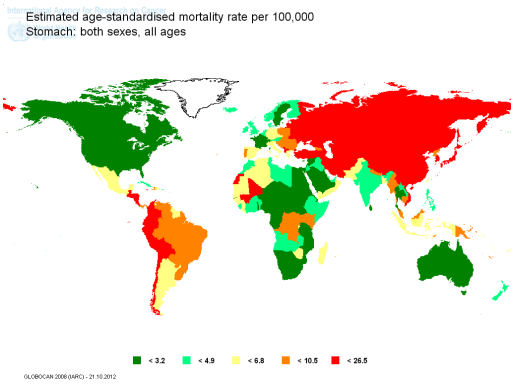


Figure 2: Stomach cancer age-adjusted mortality rates in 2008 per 100,000

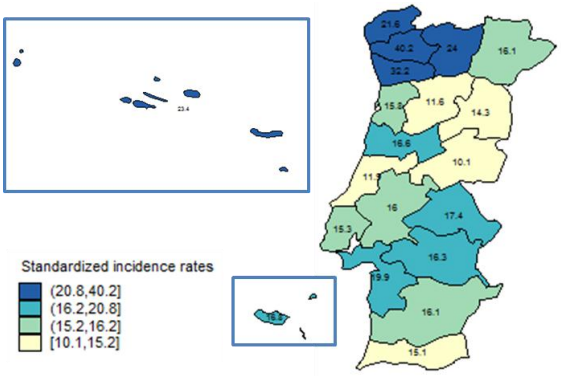


Recently, gastric cancer has been described as one of the cancers with the highest disability-adjusted life-years (DALY's), 241 in males and 146 in females, representing 20% of the total DALY's cancer burden worldwide (41).

Nevertheless, a decrease in incidence and mortality has been observed worldwide in the last decades (42-44). The decline in gastric cancer mortality was first noted in the 1930s in the United States (44). In the 1960´s almost all European countries, Australia and Japan presented mortality rates decaying (42), although not all at the same velocity. Therefore, in the 1980s, this phenomenon was called as an "unplanned triumph" (45). This decline has been attributed to an improvement in socio-economic conditions, with an increase in the consumption of fruit and vegetables and a decrease in exposure to salty food (45). Nevertheless, the variation in the frequency of *H. pylori* infection may have also strongly contributed to this "unplanned triumph".

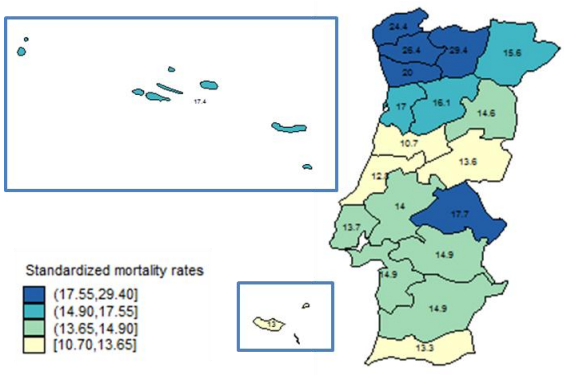
In Portugal, despite a steady decline in mortality observed over the last four decades (42, 46), gastric cancer incidence and mortality rates (47) are among the highest in Europe. The distribution of incidence and mortality is not homogeneous across regions, with the North of the country presenting higher rates (48, 49) (figure 3 and 4).

Figure 3: Stomach cancer standardized incidence rates per 100,000 (direct method, European population) in 2005*



*Data supplied by RORENO, RORSUL, RORCENTRO and RORA

Figure 4: Stomach cancer standardized mortality rates per 100,000 (direct method, European population) in 2005*



*Data from the publication:"Risco de Morrer em Portugal 2005" (49)

THE DYNAMICS AND THE GEOGRAPHICAL DISTRIBUTION OF *H. PYLORI* INFECTION

Half of the world adult population is estimated to be infected by *H. pylori* (50), and the prevalence varies appreciably across geographical areas (50-52). Ranging from 74% (overall estimate of prevalence of infection in middle-aged adults) in less developed areas to 58% in more developed areas (50). Two main patterns of *H. pylori*

prevalence in respect to age have been described. In low and middle income countries infection is acquired mainly in childhood and may reach nearly 100% during adulthood. In high income countries, the infection is less common in children and gradually increases with age (52).

The incidence of infection is higher in children than in adults (52, 53), especially in developing countries, and it has been suggested that acquisition occurs almost exclusively in the first five years of life (54). However, the annual rates of seroconversion among adults range from 0.2% to 1.1% in most populations (52), and the acquisition of infection in adulthood may not be negligible. Additionally, the annual reinfection rates after *H. pylori* eradication have been reported to be as high as 13%-24% in some low-income communities, and adults from affluent regions who visit or move to developing countries (*e.g.* backpackers, military personnel, missionaries) may seroconvert at rates similar to the observed in children from the host countries (52). The occurrence of *H. pylori* infection in adulthood may therefore be interpreted as the combined effect of a birth-cohort phenomenon and sustained acquisition throughout life (53).

Nevertheless, the comparison of the prevalence of *H. pylori* across different age groups suggests that its acquisition has been decreasing in the most recent cohorts (52, 55), or even stabilizing, in settings where the decreasing trend in adults had already began several years ago (56).

There are very few published studies evaluating *H. pylori* infection in Portugal (57-64) in asymptomatic populations (table 2). Although representing different populations, these studies consistently showed a high prevalence of infection regardless of the year/period of diagnosis, of the assay used to assess *H. pylori* infection and of the region. The Portuguese data suggest a pattern of variation with age that is similar to the one observed in low and middle income countries, with high prevalence of infection since adolescence or young adulthood and increasing throughout life. This is in accordance with the high rates of gastric cancer incidence in Portugal.

Table 2. Description of the Portuguese studies assessing the prevalence of *H. pylori* infection.

1st author, year of publication	Region	Participants selection	Year/period of diagnosis	Sample	Method to assess infection status	Age-group (years)	Prevalence (%)
Oleastro, 2011 (64)	Lisboa	844 children aged between 0 to 15 years were randomly selected, from health care centres of the Lisboa area, to be part of this cohort study	2002/2003	Stool	Immunoenzymatic	0-5	19.9
						6-10	37.0
						11-15	51.5
Rodrigues, 2001 (63)	Lisboa	583 serums from children, followed in the paediatric service of the Hospital Santa Maria, Lisboa, aged between 0.5 and 14 years were randomly selected	Not stated	Blood	ELISA	0.5-2	6.5
						3-6	19.4
						7-9	26.1
						10-14	31.4
Jardim, 2001 (62)	Madeira	375 individuals from all ages were randomly selected to participate in this study	Not stated	Expired air	Urea breath test	5-19	74.0
						20-39	82.5
						40-59	86.8
						≥60	82.5
Baldaia*, 2001 (61)	Coimbra	55 healthy controls	Not stated	Expired air	Urea breath test	<40	70.4
						≥40	100.0
Silva, 1999 (60)	Gaia	115 children, born in 1987 and 1992, living in Vila Nova de Gaia who attended 2 primary schools in this area were considered eligible to be part of this cross-sectional study	1998	Expired air	Urea breath test	6-8	45.9
						9-11	56.3

Table 2. Description of the Portuguese studies assessing the prevalence of *H. pylori* infection (continuation).

1st author, year of publication	Region	Participants selection	Year/period of diagnosis	Sample	Method to assess infection status	Age-group (years)	Prevalence (%)
Quina, 1994 (58)	Portugal	Subjects without known digestive tract pathologies from 20 hospitals in 11 Portuguese cities were part of this cross-sectional study	Not stated	Blood	ELISA	3-6	46.2
						7-9	49.1
						10-14	47.6
						15-19	70.8
						20-29	71.7
						30-39	80.0
						40-49	88.3
						50-59	87.6
						60-69	84.7
						≥ 70	90.8
EUROGAST, 1993 (59)	Gaia	132 Individuals (25-34 years and 55-64 years) participated in the EUROGAST cross-sectional study aiming to described the prevalence of infection with *H. pylori* in asymptomatic subject, from 17 geographically defined populations were evaluated	Not stated	Blood	ELISA	25-34	55.0
						55-64	70.0
Estevens, 1993* (57)	Lisboa	Eighty voluntary blood donors and patients from external consultation for trauma and orthopaedics were matched with eighty patients with gastric adenocarcinoma diagnosed in 1990 and 1991 on the Portuguese Institute of Oncology of Lisbon	1990/1991	Blood	ELISA	65.7	81.5

*Prevalence among controls.

HELICOBACTER PYLORI TRANSMISSION AND DETERMINANTS OF INFECTION

The transmission of *H. pylori* infection is still not entirely clarified. Iatrogenic contamination following endoscopy is the only proven mode (15). Waterborne transmission, probably due to faecal contamination, may be an important source of infection, especially in areas of the world with deficiencies in water treatment (15). However, for the general population, the most likely mode of dissemination is from person to person, by the oral-oral route or by the faecal-oral route (15). Intrafamilial transmission seems to be the main route of infection, and DNA typing showed that within the members of a family the same strain of the bacteria is often found (54). The finding that transmission appears to occur primarily between mothers and offspring (65) and among siblings (53) fits the hypothesis of close contact being important for the acquisition of the infection.

The relationship between low socioeconomic status and *H. pylori* infection is known for a long time (15, 59), specially socioeconomic status in the childhood, as infection is acquired predominantly at young ages (66). A poor socio-economic condition favors infection (66-68), which is frequently acquired in conditions of poor hygiene and inadequate housing standards. Also related with socioeconomic childhood conditions, a positive association has also been found with the low education of the parents (mother, or father or both) and low income (69). Height, measuring socioeconomic status during childhood has been also investigated. However, this relation is not consensual (70, 71).

The number of siblings and household overcrowding during childhood, probably reflecting the probability of close contacts with infected individuals, has also been positively associated with *H. pylori* infection (72-75). The contacts between children and their mothers and between siblings have been described as the main factors contributing to the transmission of infection in early life (53, 65, 76). The attendance to child-care centres, measuring not only close contacts with a high number of young children, but also, in some situations, the absence of appropriate hygienic practices, have also been investigated and a higher risk of infection among children attending child-care institutions has been reported (64, 77).

The role of gender in the acquisition of *H. pylori* infection has also been frequently analysed. Although controversial, male gender has been associated with an increased risk of *H. pylori* infection (78).

Controversial findings were published regarding the association between smoking and *H. pylori* infection (15). However, this relation is biologically plausible and it was also shown to contribute for the failure of *H. pylori* eradication (79). Alcohol

consumption has also been addressed by several studies but the majority of them did not find any significant association with the acquisition of the infection (15).

Several studies have also investigated the relation between *H. pylori* infection and various hygiene practice indicators (15). Showing that, generally, poor hygiene practices, especially during childhood, are risk factors for *H. pylori* infection. Some of these practices are only applied to less-developed settings and include poor bathing conditions during childhood, sharing cups as children, bad hygiene practices at home, having mothers who prechew the food for their young children, using chopsticks or living in small areas with limited sanitary facilities (15).

Ethnicity has also been evaluated. Initially the majority of the studies showed increased prevalence in immigrant groups from higher prevalence regions and in ethnic groups from lower socioeconomic status (69). Very recently, in a study from US, using genetic markers, the authors were able to predict highest prevalence rates among individuals with the highest African ancestry, after adjustment for education, socioeconomic status, and other factors. These findings suggest a possible genetic susceptibility to *H. pylori* infection (80).

PREVENTION OF *H. PYLORI* INFECTION

H. pylori infection contributes to a large proportion of gastric cancer cases worldwide and eradication may be a chemopreventive strategy to reduce gastric cancer incidence. However the effectiveness of this approach is not fully demonstrated. Experimental studies on the association between *H. pylori* infection and gastric cancer are scarce. The first and only placebo-controlled randomized study having gastric cancer as the primary outcome supporting that eradication prevents the development of gastric cancer was published in 2004 (81). In a recent meta-analysis, considering 6695 participants followed between 4 to 10 years, a protective effect on the occurrence of gastric cancer after *H. pylori* eradication was found (RR=0.65; 95% CI, 0.43 to 0.98) (82). Interestingly, none of the studies included found any statistical significant reduction in the incidence of gastric cancer after eradication, probably due to lack of statistical power, suggesting the necessity of larger studies in order to disclose the real effect of eradication on the occurrence of gastric cancer (82).

Antimicrobial therapies for *H. pylori* infection have been very successful, achieving eradication rates of more than 90% (83). However, effective treatment of an *H. pylori* infection requires multidrug regimens; and treatment must be taken several times a day for at least 7 days, which may contribute to poor adherence to the treatments. Additionally, antibiotic resistance to frequently used antibiotics is an important problem in some countries (83, 84). Moreover, the successful eradication of

H. pylori infection through antimicrobial therapy does not provide continued protection against the infection (85), as acquisition through life is possible. Therefore, widespread vaccination against *H. pylori* infection in populations with an increased risk of gastric cancer would reduce the incidence of gastric cancer without having to identify specific individuals harbouring *H. pylori* infection (86). For all these reasons, much effort has been made in the development of a vaccine against *H. pylori* infection in humans. However, it resulted in relatively few clinical trials in humans. And therefore the development of a vaccine is still in an early stage (86).

However, vaccines and antibiotics are not the only means for prevention and cure of *H. pylori*-associated disease. A better understanding of the determinants of *H. pylori* infection acquisition at different ages may contribute to a new life course framework for gastric pathology research. This information is crucial to develop preventive strategies in order to accelerate the disappearance of infection in high-prevalence populations and to increase its extension in low-prevalence settings, aiming the decrease in gastric cancer incidence and mortality.

Aims

The increasing standards of hygiene, including improved sanitation, less frequent close contacts, and increased antibiotic consumption shifted the age of *H. pylori* acquisition from early to late childhood, to adolescence and then to adulthood among consecutive generations. Therefore, the occurrence of *H. pylori* infection in adulthood may be interpreted as the combined effect of a birth-cohort phenomenon and sustained acquisition throughout life. The incidence of the different gastric outcomes of *H. pylori* infection depends on the age at acquisition, and the understanding of the determinants of *H. pylori* infection at different ages may contribute to a new life course framework for gastric pathology research, especially in high-risk settings. Therefore, this thesis aims to identify factors associated with *H. pylori* infection at different ages (distinguishing the potentially critical periods of childhood and adolescence, in addition to adulthood), through the following specific objectives:

1) To quantify the prevalence and the incidence of *H. pylori* infection and the proportion of subjects infected with CagA-positive strains in a cohort of adults (EPIPorto) and to identify the major sociodemographic correlates of the infection (study I).
2) To quantify the prevalence and the incidence of *H. pylori* infection in a cohort of adolescents (EPITeen) and to identify risk factors for the infection (study II).
3) To quantify the prevalence of *H. pylori* infection in a cohort of children evaluated at ages between 4 and 5 years (Geração XXI), and to assess the independent effect of child-care attendance in the early life acquisition of the infection (study III).
4) To quantify the association between child-care attendance and *H. pylori* infection through systematic review and meta-analysis (study IV).

RESEARCH METHODS

The objectives of this thesis were accomplished through the analysis of data obtained from three Portuguese population-based cohorts (EPIPorto, EPITeen and Geração XII) and through a systematic review of the published literature. The selection of participants to be used in each analysis depended on the specific objectives of the investigations and is described in detail in the methods section of each one of the papers.

Participants selection

Studies I, II and III were based on three cohort samples established in the city of Porto. Study I was based on a subsample of participants of the EPIPorto cohort, a cohort sample of adults, assembled in order to evaluate health determinants of non-institutionalised adult dwellers in the city of Porto. Participants were recruited between January 1999 and December 2003, by random digit dialling using households as the sampling frame, followed by simple random sampling to select one eligible person among permanent residents in each household, without allowing replacement of refusals. Selected participants were invited to visit the Department of Clinical Epidemiology, Predictive Medicine and Public Health to a face-to-face interview and to perform a physical examination. The EPIPorto cohort included 2485 participants aged above 18, corresponding to a participation proportion of 70%. A second evaluation was performed between 2005 and 2008 and the participation rate was approximately 68% (median time of follow-up: 5 years).

Study II was based on a sample of adolescents of the EPITeen cohort, a representative sample of adolescents born in 1990 constituted in order to study growth, development and health from adolescence to young adulthood. The participants were recruited in the schools from Porto, Portugal, in 2003/2004. The executive boards of all schools from Porto teaching the 9th grade (27 public and 24 private) were asked to provide the addresses of students aged 13 years. All public schools and 19 (79%) of the private schools allowed the research team to contact the eligible students and their families. In compliant schools, 2787 eligible adolescents were identified (2126 in public and 661 in private schools) and 2160 (1651 public and 509 private school students) provided information at least for part of the planned assessment. This resulted in a 77.5% overall participation proportion, similar in public and private schools. The second evaluation of this cohort was conducted between January 2007 and May 2008, and 1716 (79.4%) participants were re-evaluated. The third evaluation started in 2011 and is on-going.

Study III was part of the cohort Geração XXI, the first prospective Portuguese population-based birth cohort, assembled in Porto in the North of Portugal. All mothers resident in the metropolitan area of Porto, who delivered a live-born baby between April 2005 and September 2006 in one of the five public hospitals with maternity, were invited to participate. The participation rate among the mothers was 91.4% and a total of 8647 children were enrolled in the cohort. Between April 2009 and April 2011, all the children, aged between 4 and 5 years old (median time of follow-up: 50 months), were invited to attend the re-evaluation of the cohort and 86.2% of the children and 84.2% of the mothers were re-evaluated. A re-evaluation of the children at 7 years-old started in 2012 and is on-going.

H. pylori infection assessment

In studies I, II and III the assessment of anti-*Helicobacter pylori* IgG titres on serum was performed by ELISA [Anti-*Helicobacter pylori* ELISA (IgG), Euroimmun, Lubeck, Germany]. The participants' infection status was classified as negative if the antibody concentration was lower than 16 RU/ml, borderline if the antibody concentration was equal or higher than 16 RU/ml and lower than 22 RU/ml, and positive if the antibody concentration was 22 RU/ml or higher. For analysis, subjects having borderline results were considered infected.

In study I, a subsample of participants classified as infected according to the anti-*Helicobacter pylori* IgG titres was also tested by Western Blot (Helico Blot 2.1, Genelabs Diagnostics®, Singapore) in order to achieve prevalence of infection with CagA-positive strains. CagA seropositivity was evaluated following the criteria recommended by the manufacturer: presence of the 116kD band (CagA) with one or more of the following bands: 89kD (VacA); 37kD; 35kD; 30kD (UreA) and 19.5kD together.

The systematic review and meta-analysis

In order to quantify the association between child-care attendance and *H. pylori* infection, PubMed® was searched from inception to July 2012. The evaluation of the literature was performed by two reviewers independently, following a three-step predefined protocol. The decisions taken by the two researchers were compared in all steps and the discrepancies were discussed until consensus or resolved involving a third researcher. The literature search was further complemented by backward citation tracking among the articles considered eligible for the systematic review. The same two reviewers independently extracted the information, and discrepancies in the data extracted were discussed and resolved by consensus, or involving a third researcher

whenever necessary. In order to compute summary estimates of the association between child-care attendance and *H. pylori* infection a meta-analysis was performed.

PAPERS

PAPER I

Socio-demographic determinants of prevalence and incidence of *Helicobacter pylori* infection in Portuguese adults.

Abstract

Sociodemographic determinants of prevalence and incidence of *Helicobacter pylori* infection in Portuguese adults

Objectives:

Understanding the determinants of *H. pylori* infection in adults is essential to predict the burden of *H. pylori*-related diseases. We aimed to estimate the prevalence and incidence of *H. pylori* infection and to identify its major sociodemographic correlates in an urban population from the North of Portugal.

Methods:

A representative sample of non-institutionalised adult inhabitants of Porto (n=2067) was evaluated by ELISA (IgG) and a subsample (n=412) was tested by Western Blot to assess infection with CagA-positive strains. Modified Poisson and Poisson regression models were used to estimate crude and sex-, age-, and education-adjusted prevalence ratios (PR) and incidence rate ratios (RR), respectively.

Results:

The prevalence of *H. pylori* infection was 84.2% [95% confidence interval (95%CI): 82.4-86.1]. It increased across age groups in the more educated subjects, (18-30 years: 72.6%; ≥71 years: 88.1%; P for trend<0.001) and decreased with education in the younger (≤4 schooling years: 100.0%; ≥10 schooling years: 72.6%; P for trend<0.001). Living in a more deprived neighbourhood was associated with a higher prevalence of infection, only in the younger (PR=1.20, 95%CI: 1.03-1.38) and more educated participants (PR=1.15, 95%CI: 1.03-1.29). Among the infected, the proportion with CagA-positive strains was 61.7% (95%CI: 56.6-66.9). The incidence rate was 3.6/100 person-years (median follow-up: 3 years; 95%CI: 2.1-6.2), lower among the more educated (≥10 *vs.* ≤9: RR=0.25, 95%CI: 0.06-0.96). The seroreversion rate was 1.0/100 person-years (95%CI: 0.6-1.7).

Conclusion:

The prevalence of infection among adults is still very high in Portugal, suggesting that stomach cancer mortality rates will remain high over the next few decades.

Introduction

Helicobacter pylori infection is strongly associated with the occurrence of gastric cancer (1), and individuals who are infected with CagA-positive strains are at an even higher risk than those harbouring strains without this virulence marker (2).

The frequency of the infection varies appreciably with age and across geographical areas (3). It is acquired mainly in childhood, though infection may also occur in adult life (4) and is observed more often among subjects with lower socioeconomic status (5). In the latest decades, the prevalence of infection in adult populations declined to below 50% in the more affluent European countries (3), contributing to the steep decline in gastric cancer incidence and mortality (6). Nevertheless, approximately half the world adult population is infected (7) and about 660,000 of all cancers diagnosed in 2008 worldwide were attributable to *H. pylori* infection, corresponding to approximately one third of the 2 million cancer cases due to infections (8).

In Portugal, despite a steady decline in mortality over the last four decades (6, 9), gastric cancer rates (10) are amongst the highest in Europe, especially in the North of the country (11), where this study was conducted. The prevalence of *H. pylori* infection in the Portuguese adult population was approximately 90% in the early 1990s (12); to our knowledge, no surveys of the adult general population were conducted since then, and the available data on the frequency of infection with the more virulent strains is scarce (13, 14).

We aimed to estimate the prevalence and the incidence of *H. pylori* infection and the proportion of subjects infected with CagA-positive strains, and to identify the major sociodemographic correlates of infection in an urban population from the North of Portugal.

Methods

Non-institutionalised adult dwellers in the city of Porto were recruited between January 1999 and December 2003 and re-evaluated between May 2005 and September 2008, as part of the EPIPorto cohort. A detailed description of the general selection procedures and participants' characteristics has been published elsewhere (15, 16). In brief, participants were recruited by random digit dialling using households as the sampling frame, followed by simple random sampling to select one eligible person among permanent residents in each household, without allowing replacement of refusals. The participants underwent a questionnaire evaluation, physical examination and blood collection at the Department of Clinical Epidemiology, Predictive Medicine and Public Health of the University of Porto Medical School, Porto, Portugal.

A flowchart describing the participants considered for the different components of the present study is presented in figure 1.

The EPIPorto cohort included 2485 participants aged above 18, corresponding to a participation proportion of 70% (16). A venous blood sample was available for 2067 subjects (83.2%). Participants from whom a blood sample was not available were older (median age: 58 *vs.* 55 years, P<0.001) and less educated (median number of education years: 6 *vs.* 8 years, P<0.001). The groups were similar regarding sex distribution (males: 39.5% *vs.* 37.8%, P=0.529).

To assess the incidence of infection during follow-up, a venous blood sample was collected from 114 participants (40.6% of those without evidence of infection at the baseline evaluation; median follow-up: 3 years). Participants testing negative for infection at baseline from whom a blood sample was not available at follow-up were significantly older (median age: 54 *vs.* 41 years, P<0.001) and less educated (median number of education years: 11 *vs.* 12 years, P=0.031). The sex distribution was not significantly different between these two groups (males: 31.7% *vs.* 36.8%, P=0.374).

To quantify the seroreversion rate, a random representative sample, stratified according to sex, age and education, of individuals classified as infected at baseline and reevaluated at follow-up (n=261, median follow-up: 5 years) was obtained.

Serum samples were kept frozen at -20ºC until analysis. Assessment of anti-*Helicobacter pylori* IgG titres on serum was performed by ELISA [Anti-Helicobacter pylori ELISA (IgG), Euroimmun, Lubeck, Germany; Sensitivity: 100%, Specificity: 94% (17)]. The participants' infection status was classified as negative if the antibody concentration was lower than 16 RU/ml, borderline if the antibody concentration was equal or higher than 16 RU/ml and lower than 22 RU/ml, and positive if the antibody

concentration was 22 RU/ml or higher. For analysis, subjects having borderline results were considered infected.

To quantify the prevalence of infection with CagA-positive strains at baseline, a subsample of 412 participants classified as infected according to the anti-*Helicobacter pylori* IgG titres, randomly selected to be representative of the infected individuals at the baseline regarding sex, age and education, was further tested by Western Blot (Helico Blot 2.1, Genelabs Diagnostics®, Singapore). CagA seropositivity was evaluated following the criteria recommended by the manufacturer: presence of the 116kD band (CagA) with one or more of the following bands: 89kD (VacA); 37kD; 35kD; 30kD (UreA) and 19.5kD together.

Education was recorded as completed years of schooling and further categorized for analysis. Occupations were classified by major professional groups, according to the National Classification of Occupations - version 1994 (NCO-94) (18) and grouped in three categories: upper white collar, lower white collar and blue collar. The upper white collar category comprised individuals classified in the upper three major groups of the NCO-94: executive civil servants, industrial directors and executives; professionals and scientists and middle management and technicians. The lower white collar category comprised individuals classified in the fourth and fifth major groups of the NCO-94: administrative and related workers and service and sales workers. The blue collar category comprised individuals classified in the sixth to ninth major groups of the NCO-94. These major groups included farmers and skilled agricultural, fisheries workers, skilled workers, craftsmen and similar, machine operators and assembly workers and unskilled workers. Retired participants were classified considering their previous main occupation. Similarly, housewives and currently unemployed subjects reporting a previous occupation were included in the analysis using this information (19). Housewives and unemployed at the time of data collection who did not report a previous occupation were included in an additional category, named “Other”.

The assessment of the neighbourhood’s socioeconomic class has been thoroughly described elsewhere (20). Briefly, statistical census tracts defined by the National Census 2001, broadly equivalent to a city block in an urban setting, were used as a proxy of neighbourhoods (21). Self-reported addresses were used to georeference individuals to a specific neighbourhood. The socioeconomic characterization of neighbourhoods was based on aggregated data from the National Census 2001, available at the statistical subsection level. Based on statistical criteria and consensus between investigators, 11 variables related to buildings, households, families and individuals were selected in order to characterize three distinct socioeconomic

dimensions of the neighbourhood: age, education/occupation and housing characteristics. Latent class analysis models were used to uncover socioeconomically heterogeneous and discrete groups of neighbourhoods (22). The final model identified three socioeconomic classes. Whereas class 1 neighbourhoods had the better socioeconomic position, classes 2 and 3 had increasingly worse socioeconomic profiles.

Height was measured to the nearest centimetre in the standing position using a wall stadiometer. For analysis, the participants' height was categorized according to the sex-specific tertiles of its distribution.

Information on refrigerator ownership was collected by asking the subjects if they had refrigerator at home, and for how many years. For analysis the participants were classified as having a refrigerator during all, or only part of their lives.

Proportions were compared using the χ^2 test, or Fisher's exact test, when appropriate. Trends across ordered groups were analysed using the χ^2 test for trends.

The modified Poisson regression (23), with a robust estimator of the standard error, and Poisson regression were used to estimate sex-, age-, and education-adjusted prevalence ratios (PR) and incidence rate ratios (RR), respectively, with the corresponding 95% confidence intervals (CI). To compute the incidence rate and the RR estimates, the time at risk was defined as the difference between the date of the second evaluation and the date of the first evaluation when participants were not infected at follow-up, or half of this difference for participants infected during the follow-up. To estimate the seroreversion rate, the time at risk was defined as the whole follow-up if the participants remained infected, or half this time for those that seroreverted.

All analyses were conducted using STATA®, version 9.2, considering sampling weights to account for differences between our sample and the known age and sex structure of the population of Porto (24).

Results

Prevalence of *H. pylori* infection

At baseline, the prevalence of *H. pylori* infection was 84.2% (95%CI: 82.4%-86.1%). It increased with age in the subjects with ≥10 schooling years (from 72.6% in the age-group 18-30 years to 88.1% in those aged ≥71 years, P for trend<0.001), and a decline was observed among those with ≤4 schooling years (from 100.0% in the age-group 18-30 years to 90.2% in the participants aged ≥71 years, P for trend<0.001). The prevalences were lower in the more educated subjects, though the differences across the levels of education decreased with age, from nearly 30% in the younger to less than 5% in the older (figure 2).

As shown in table 1, the association between most of the participants' sociodemographic characteristics and *H. pylori* infection differed significantly across age and education groups.

Among individuals aged 18-40 years, the more educated (≥10 *vs.* ≤4 schooling years: adjusted PR=0.74, 95%CI: 0.66-0.83) and those having refrigerator all their lives (adjusted PR=0.78, 95%CI: 0.67-0.91) were less likely to be infected, while those living in more deprived neighbourhoods were more likely to be infected (most *vs.* least deprived: adjusted PR=1.20, 95%CI: 1.03-1.38). In subjects aged 41-60 years, the more educated were also significantly less likely to be infected (≥10 *vs.* ≤4 schooling years: adjusted PR=0.86, 95%CI: 0.82-0.91) and those living in more deprived neighbourhoods were more frequently infected (most *vs.* least deprived: adjusted PR=1.14, 95%CI: 1.07-1.22). In the group of participants aged above 60 years, infection was more frequent in males (adjusted PR=1.06, 95%CI: 1.01-1.11).

Among the more educated subjects, the prevalence of infection increased with age (61-92 vs. 18-40 years: adjusted PR=1.21, 95%CI: 1.10-1.33); the participants living in more deprived neighbourhoods (most *vs.* least deprived: adjusted PR=1.15, 95%CI: 1.03-1.29) or having blue-collar occupations (blue-collar *vs.* upper white-collar: PR=1.18, 95%CI: 1.02-1.37) were also more likely to be infected.

Among the participants with ≤4 years of schooling, those having refrigerator all their lives were more likely to be infected (adjusted PR=1.09, 95%CI: 1.04-1.16), while an inverse association was observed in those with 5-9 years of schooling (adjusted PR=0.89, 95%CI: 0.81-0.97).

No association was found between height and *H. pylori* infection across strata of age or education.

Prevalence of infection with CagA-positive strains, among the *H. pylori*-infected

In the subsample of infected subjects that were evaluated by Western Blot, the prevalence of infection with CagA-positive strains was 61.7% (95%CI: 56.6%-66.9%). The more educated were less likely to be infected with these high-virulence strains (≥10 *vs.* ≤9 schooling years: adjusted PR=0.81, 95%CI: 0.67-0.99) (table 2).

Incidence of *H. pylori* infection and seroreversion

The incidence rate was 3.6/100 person-years (95%CI: 2.1-6.2). The more educated participants had a lower risk of infection (≥10 *vs.* ≤9 schooling years: adjusted RR=0.25, 95%CI: 0.06-0.96) (table 3).

The seroreversion rate was 1.0/100 person-years (95%CI: 0.6-1.7).

Discussion

Our study shows a high prevalence of *H. pylori* infection in urban Portuguese adults evaluated between 1999 and 2003, increasing across age groups in the more educated subjects and decreasing with education in the younger. Living in a more deprived neighbourhood was associated with a higher risk of infection, only among the younger and more educated participants.

Although this is the first study assessing the prevalence and incidence of *H. pylori* infection in the general population of Porto, the comparison of our results with estimates published in 1994 for the overall Portuguese population (approximately 84% in individuals aged above 19 years) (12) suggest that the prevalence did not change in the last decade. Therefore, this important risk factor for gastric cancer remains frequent in this setting. Furthermore, the prevalence observed in our study is likely to underestimate the true prevalence, especially among the young subjects, because the participants evaluated for infection status tended to be more educated than the remaining. Our estimates are among the highest observed in the world (7), close to those observed in some Eastern European and South American countries (*e.g.* 82.0% in Brazil in 2010 (25); 88.0% in Russia in 1996 (3); 86.0% in a Cape Verdean immigrants in The Netherlands in 2004 and 2005 (26)). This is in accordance with the high gastric cancer incidence and mortality rates in Portugal, especially in the North (9, 11), and with the fact that the decline in mortality only started in the mid-1970s, later than in most developed countries (27).

In the last half century Portugal went through several important economical and societal changes. After the Second World War the country evolved from a rural to a service economy, and in the 1970s the political system changed from a dictatorial regime to a political democracy. These transitions resulted in an improvement of the living standards, although unequally across the different socioeconomic groups (28). The pace of these historical changes contributes to understand the currently high prevalence of infection and the distinct patterns depicted by our results. The comparison of the prevalence of *H. pylori* across different age groups suggests that its acquisition has been decreasing in the most recent cohorts, in accordance with observations from other high-income countries where prevalence of infection is low (*e.g.* United Kingdom, The Netherlands, Finland), intermediate (*e.g.* Greece), or high (*e.g.* Japan) (4), although in our study this pattern only applies to the more educated subjects. This likely reflects general improvements in hygiene practices over time (29), and is also in accordance with the higher prevalence observed in the participants living in more deprived neighbourhoods only among the younger and the more educated. On

the other hand, among the participants with a lower education level the age-specific prevalences resemble more closely the observed in low-and-middle-income countries characterized by high prevalences since young adulthood, and possibly lower prevalences among the older subjects due to the high proportion of subjects with chronic atrophic gastritis (30) and intestinal metaplasia (31), and consequent spontaneous disappearance of the infection.

Height may be used, on a population level, as an indicator of growth, nutrition and social environment in early life, and can also measure socioeconomic status during childhood (32). In our data, *H. pylori* infection was not associated with height, in accordance with previous observations of no significant independent association between adult height and *H. pylori* infection (33). However, this relation is not consensual. A study from 2005 found an association between adult height and *H. pylori* infection, but only among women (34); however, the authors did not discard the hypothesis of residual confounding due to the small effect size and due to the cross-sectional study design. A more recent study found a modest increase in the risk of *H. pylori* infection with height (OR per meter: 0.05; 95% CI: 0.01–0.24) (35).

The availability of a refrigerator reflects both the living conditions in childhood and socioeconomic status. Refrigerators became available in most Portuguese families between the late 1970's and the early 1980's (36) and its ownership was initially restricted to the higher socio-economic classes. This exposure is therefore a surrogate for socioeconomic status, but also for the less frequent consumption of salted foods. However, the results were not consistent across the different strata analysed and are not easily interpretable.

A meta-analysis of population-based prevalence surveys showed a higher prevalence among men (OR=1.16, 95%CI: 1.11-1.22) (37). Since there is no evidence of sex differences in the acquisition of the infection at young ages, this may be explained by a greater loss of infection among females, possibly due to a greater female exposure to antibiotic treatments throughout life (37, 38). This hypothesis is compatible with our observation of a slightly higher prevalence of infection in men only among the elderly.

CagA seropositivity has been associated with a substantially increased risk of gastric cancer (2), and CagA-positive strains have been reported to account for 60% to 80% of the infections in Portugal and in other European countries (13, 39). In a recent study from US, subjects living in neighbourhoods with higher house values and where proportionately more adults had a high school education and were employed had lower odds of being sero-positive for CagA-positive strains than those living in neighbourhoods with lower levels of these socio-economic status measures (40).

Despite the overall high prevalence of infection observed in our study, we also showed a tendency for more educated participants and those living in less deprived neighbourhoods to have a lower prevalence of infection with the more virulent strains, which may reflect a lower frequency of multiple infections among subjects with a higher socioeconomic status (41).

A recent systematic review (42) showed annual incidence rates of *H. pylori* infection among adults below 1.0% in 17 out of 32 studies and between 1.0% and 3.0% in most of the remaining. However, measurement error could account for the apparent cumulative incidence estimates observed in these studies, even in the absence of any true new infections. When applying the most stringent criteria used in the previous report to define incident cases of infection (excluding the participants with borderline results at baseline and considering that a new infection occurred only if there was an increase of at least 4-fold in the antibody concentrations from baseline to follow-up), the incidence rate in our study would be 2.0/100 person-years (95%CI: 1.1-4.5). The high rate observed even when the specificity of the test used to assess the infection status was further improved, as well as the significantly lower incidence rate observed among the more educated participants, strengthen our conclusions of a high incidence of infection in this adult population. Furthermore, the infection at the second evaluation was assessed for only 41% of individuals not infected at baseline, and those not evaluated tended to be older and less educated, contributing to underestimate the incidence. In our study the seroreversion rate was similar to the observed in other developed countries (*e.g.* Japan, 0.7/100 person-years (43), Denmark, 0.8/100 person-years (44)). Although we considered participants with borderline results as positive for infection, sensitivity analyses excluding individuals with borderline IgG titres or considering borderline results as negative yielded no meaningful differences (data not shown). We also compared antibody concentration of individuals that seroreverted in successive evaluations. Two of these 14 participants had a borderline result in the first evaluation, and the other 12 had IgG titres much higher than the cut off for positivity (median antibody concentration: 76.5 RU/ml) and none of these individuals presented a borderline result at the second evaluation (median antibody concentration: 12.1 RU/ml). Therefore, these results are more likely to reflect a previous infection that was resolved than misclassification. Unfortunately, the small number of participants in which seroreverter was observed does not allow the assessment of its determinants.

In conclusion, our results show that the prevalence of *H. pylori* infection in Portugal remains in the upper bound of those observed across European countries. Stomach cancer incidence and mortality rates are likely to continue amongst the highest in Europe during the next decades, particularly in less educated individuals.

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Table 1. Association between sociodemographic characteristics and prevalence of infection, defined by the titres of anti-*Helicobacter pylori* serum IgG*.

		According to the participants' age (years)							According to the participants' education (years)						
	All participants	18-40		41-60		61-92			≤4		5-9		≥10		
	% (95% CI)	%	Adjusted† PR (95% CI)	%	Adjusted† PR (95% CI)	%	Adjusted† PR (95% CI)	P‡	%	Adjusted§ PR (95% CI)	%	Adjusted§ PR (95% CI)	%	Adjusted§ PR (95% CI)	P‡
Sex															
Female	83.7 (81.4-85.9)	75.0	1.00 [ref.]‖	88.8	1.00 [ref.]‖	87.5	1.00 [ref.]‖		91.5	1.00 [ref.]¶	87.6	1.00 [ref.]¶	75.8	1.00 [ref.]¶	
Male	85.0 (81.9-88.0)	75.6	1.00 (0.89-1.13)	89.3	1.02 (0.97-1.07)	92.2	1.06 (1.01-1.11)	0.371	94.2	1.03 (0.99-1.08)	90.0	1.04 (0.96-1.11)	77.6	1.02 (0.93-1.11)	0.992
Age (years)															
18-40	75.3 (71.0-79.6)	---	---	---	---	---	---		95.6	1.00 [ref.]**	84.6	1.00 [ref.]**	71.2	1.00 [ref.]**	
41-60	89.0 (86.9-91.1)	---	---	---	---	---	---		95.0	0.99 (0.90-1.08)	91.8	1.09 (0.98-1.20)	82.4	1.16 (1.06-1.26)	
61-92	89.5 (87.4-91.6)	---	---	---	---	---	---		90.4	0.94 (0.86-1.03)	90.4	1.07 (0.96-1.19)	86.1	1.21 (1.10-1.33)	0.008
Education (years)††															
≤4	92.4 (90.5-94.3)	95.6	1.00 [ref.]**	95.0	1.00 [ref.]**	90.4	1.00 [ref.]**		---	---	---	---	---	---	
5-9	88.8 (85.5-92.1)	84.6	0.88 (0.77-1.01)	91.8	0.96 (0.92-1.01)	90.4	0.99 (0.94-1.05)		---	---	---	---	---	---	
≥10	76.6 (73.3-80.0)	71.2	0.74 (0.66-0.83)	82.4	0.86 (0.82-0.91)	86.1	0.94 (0.88-1.01)	0.008	---	---	---	---	---	---	
Occupation‡‡															
Upper white-collar	79.3 (75.8-82.8)	69.1	1.00 [ref.]	84.9	1.00 [ref.]	90.3	1.00 [ref.]		90.2	1.00 [ref.]	92.7	1.00 [ref.]	77.2	1.00 [ref.]	
Lower white-collar	84.0 (80.3-87.8)	78.6	1.10 (0.93-1.31)	86.1	0.95 (0.87-1.04)	87.2	0.91 (0.83-1.00)		91.8	1.03 (0.88-1.21)	87.3	0.96 (0.89-1.04)	74.1	0.97 (0.86-1.10)	
Blue-collar	91.5 (89.7-93.9)	87.9	1.17 (0.97-1.41)	95.0	1.01 (0.93-1.10)	90.6	0.94 (0.87-1.03)		92.6	1.04 (0.90-1.21)	89.0	1.01 (0.92-1.10)	89.2	1.18 (1.02-1.37)	
Other	80.3 (74.9-87.0)	72.0	1.04 (0.87-1.24)	95.3	1.05 (0.95-1.16)	88.6	0.95 (0.86-1.06)	0.006	92.5	1.06 (0.90-1.25)	86.4	0.95 (0.82-1.10)	73.5	1.02 (0.88-1.18)	<0.001
Neighbourhood§§															
1 (the least deprived)	81.2 (77.2-85.2)	73.5	1.00 [ref.]**	84.5	1.00 [ref.]**	88.7	1.00 [ref.]**		92.7	1.00 [ref.]**	89.9	1.00 [ref.]**	75.7	1.00 [ref.]**	
2	83.6 (81.0-86.2)	71.8	0.97 (0.84-1.13)	89.2	1.06 (0.99-1.13)	89.1	1.01 (0.94-1.08)		91.5	0.99 (0.92-1.05)	88.5	0.98 (0.90-1.07)	75.3	0.99 (0.90-1.10)	
3 (the most deprived)	91.0 (87.6-94.4)	88.3	1.20 (1.03-1.38)	96.3	1.14 (1.07-1.22)	89.0	1.00 (0.92-1.09)	0.021	93.0	1.00 (0.93-1.07)	91.0	1.00 (0.90-1.12)	87.5	1.15 (1.03-1.29)	0.186
Height‖ ‖¶¶															
1st third	89.0 (86.3-91.6)	80.5	1.00 [ref]	92.4	1.00 [ref]	89.5	1.00 [ref]		92.7	1.00 [ref]	90.0	1.00 [ref]	79.8	1.00 [ref]	
2nd third	85.9 (83.0-88.8)	80.6	1.03 (0.88-1.21)	87.5	0.98 (0.92-1.03)	89.2	1.00 (0.94-1.05)		90.8	0.97 (0.93-1.02)	90.2	1.01 (0.93-1.09)	80.1	1.03 (0.91-1.15)	
3rd third	79.4 (75.8-83.0)	72.1	0.94 (0.80-1.10)	87.2	0.99 (0.94-1.06)	90.0	1.02 (0.96-1.08)	0.216	94.5	1.01 (0.96-1.06)	85.9	0.97 (0.88-1.08)	74.2	0.97 (0.86-1.09)	0.232
Refrigerator ownership***															
Not during all their lives	91.3 (88.3-94.3)	100.0	1.00 [ref.]	89.4	1.00 [ref.]	91.6	1.00 [ref.]		91.4	1.00 [ref.]	96.5	1.00 [ref.]	84.8	1.00 [ref.]	
During all their lives	76.6 (71.7-81.4)	72.8	0.78 (0.67-0.91)	84.5	0.99 (0.90-1.09)	100.0	1.10 (1.04-1.17)	<0.001	100.0	1.09 (1.04-1.16)	82.6	0.89 (0.81-0.97)	72.8	0.98 (0.84-1.14)	<0.001

CI-confidence interval, PR-prevalence ratio.

* For analysis, subjects having borderline results were considered infected.

† Adjusted for sex and education, except if otherwise specified.

‡ P for interaction.

§ Adjusted for sex and age, except if otherwise specified.

‖ Only adjusted for education.

¶ Only adjusted for age.

** Only adjusted for sex.

†† n=2064 due to missing data.

‡‡ n=2063 due to missing data.

§§ n=1928 due to missing data.

‖‖ n=2046 due to missing data.

¶¶ 1st third: < 153.1 cm, 2nd third: 153.1-158.3 cm, 3rd third: > 158.3 cm for females; 1st third: < 166.1 cm, 2nd third: 166.1-172.0 cm, 3rd third: > 172.0 cm for males.

*** n=704 due to missing data.

Table 2. Association between sociodemographic characteristics and infection with *Helicobacter pylori* CagA-positive strains, among *H. pylori*-infected subjects*.

	Infection with CagA-positive strains		
	%	PR (95% CI)	PR (95% CI)†
Sex			
Female	61.4	1.00 [ref.]	1.00 [ref.]‡
Male	62.0	1.01 (0.85-1.19)	1.01 (0.84-1.18)
Age (years)			
18-40	55.5	1.00 [ref.]	1.00 [ref.]§
41-92	65.0	1.17 (0.94-1.45)	1.06 (0.84-1.34)
Education (years)			
≤9	67.6	1.00 [ref.]	1.00 [ref.]‖
≥10	53.6	0.79 (0.66-0.96)	0.81 (0.67-0.99)
Occupation			
Upper white-collar	61.0	1.00 [ref.]	1.00 [ref.]
Lower white-collar	64.2	1.05 (0.83-1.33)	0.99 (0.71-1.20)
Blue-collar	65.3	1.07 (0.88-1.30)	0.89 (0.69-1.14)
Other	48.2	0.79 (0.55-1.14)	0.75 (0.51-1.09)
Neighbourhood¶			
1 (the least deprived)	54.5	1.00 [ref.]	1.00 [ref.]**
2	64.5	1.18 (0.94-1.48)	1.18 (0.94-1.49)
3 (the most deprived)	65.6	1.20 (0.92-1.56)	1.20 (0.93-1.56)
Height††			
1st third	61.4	1.00 [ref.]	1.00 [ref.]
2nd third	60.3	0.98 (0.80-1.20)	1.04 (0.86-1.27)
3rd third	63.4	1.03 (0.84-1.26)	1.18 (0.95-1.47)
Refrigerator availability‡‡			
Not during all their lives	69.0	1.00 [ref.]	1.00 [ref.]
During all their lives	54.5	0.79 (0.62-1.01)	0.81 (0.62-1.06)

CI-confidence interval, PR-prevalence ratio.

* CagA seropositivity was evaluated following the criteria recommended by the manufacturer: presence of the 116kD band (CagA) with one or more of the following bands: 89kD (VacA); 37kD; 35kD; 30kD (UreA) and 19.5kD together.

† Adjusted for sex, age and education, except if otherwise specified.

‡ Only adjusted for age and education.

§ Only adjusted for sex and education.

‖ Only adjusted for sex and age.

¶ n=377 due to missing data.

** Only adjusted for sex.

†† 1st third: < 153.1 cm, 2nd third: 153.1-158.3 cm, 3rd third: > 158.3 cm for females; 1st third: < 166.1 cm, 2nd third: 166.1-172.0 cm, 3rd third: > 172.0 cm for males.

‡‡ n=215 due to missing data.

Table 3. Association between sociodemographic characteristics and incidence of *Helicobacter pylori* infection*.

			Incidence of *Helicobacter pylori* infection		
	N	**Person-years**	**n**	**RR (95% CI)**	**RR (95% CI)†**
Sex					
Female	72	237	10	1.00 [ref.]	1.00 [ref.]‡
Male	42	162	7	1.00 (0.35-2.85)	0.79 (0.29-2.18)
Age (years)					
18-40	56	183	5	1.00 [ref.]	1.00 [ref.]§
41-92	58	216	12	2.28 (0.78-6.65)	1.33 (0.34-5.21)
Education (years)					
≤9	39	139	11	1.00 [ref.]	1.00 [ref.]‖
≥10	75	260	6	0.23 (0.08-0.66)	0.25 (0.06-0.96)
Occupation					
Upper white-collar	53	177	7	1.00 [ref.]	1.00 [ref.]
Lower white-collar	32	118	8	3.01 (0.91-9.95)	1.87 (0.54-6.60)
Blue-collar	16	58	4	2.71 (0.68-10.75)	1.20 (0.16-8.82)
Other	13	46	0	-	-
Neighbourhood¶					
1 (the least deprived)	38	141	4	1.00 [ref.]	1.00 [ref.]**
2	53	171	10	1.90 (0.56-6.47)	1.91 (0.56-6.47)
3 (the most deprived)	14	53	2	2.18 (0.36-13.11)	2.22 (0.41-12.0)
Height††‡‡					
1st third	31	119	8	1.00 [ref.]	1.00 [ref.]
2nd third	30	118	2	0.21 (0.04-1.01)	0.34 (0.06-1.93)
3rd third	50	148	7	0.84 (0.30-2.39)	2.19 (0.62-7.76)
Refrigerator availability§§					
Not during all their lives	15	36	0	1.00 [ref.]	1.00 [ref.]
During all their lives	43	105	3	-	-

CI-confidence interval, RR-incidence rate ratio.

* For analysis, subjects having borderline results were considered infected.

† Adjusted for sex, age and education, except if otherwise specified.

‡ Only adjusted for age and education.

§ Only adjusted for sex and education.

‖ Only adjusted for sex and age.

¶ n=105 due to missing data.

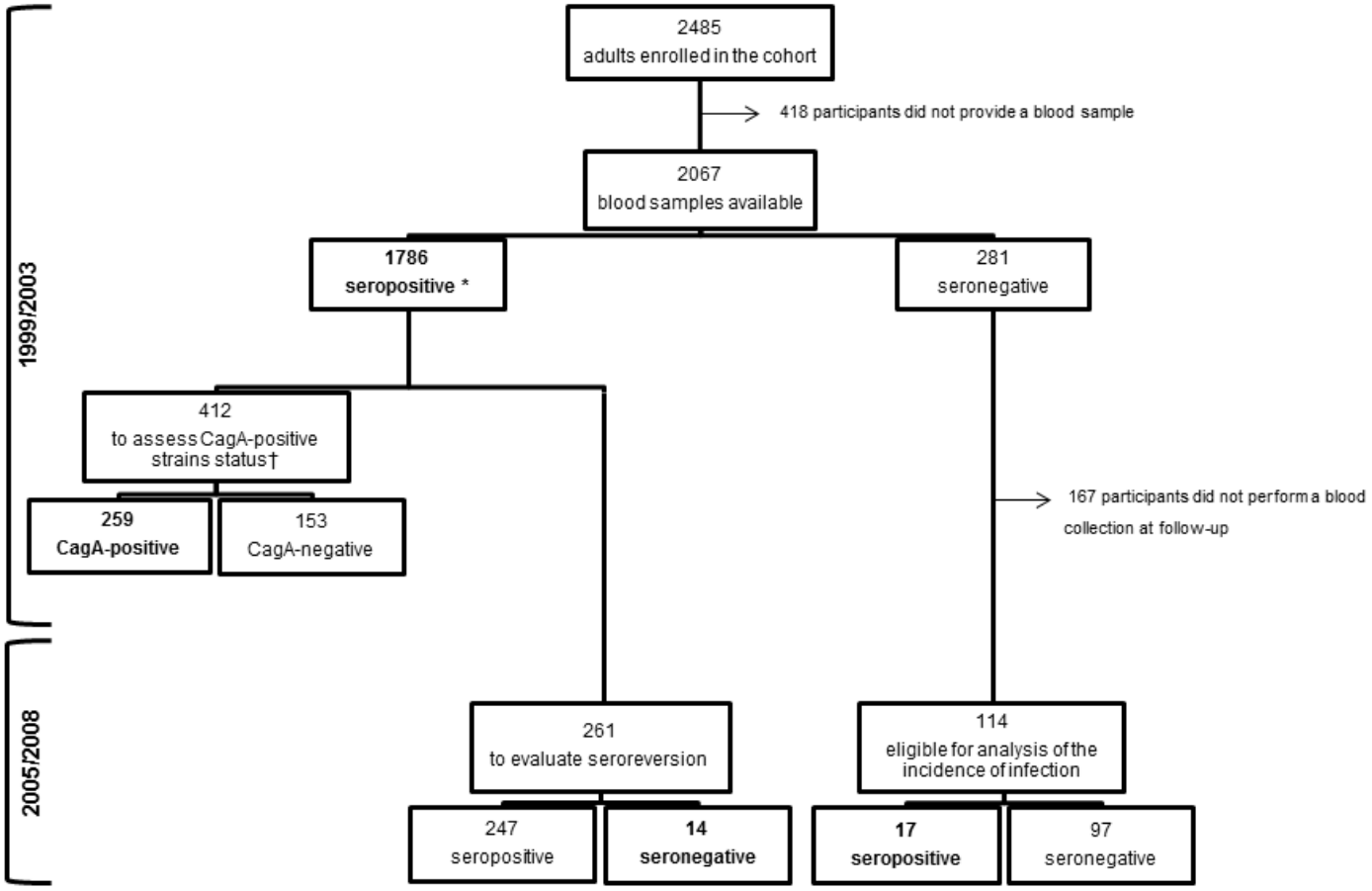
** Only adjusted for sex.

†† 1st third: < 153.1 cm, 2nd third: 153.1-158.3 cm, 3rd third: > 158.3 cm for females; 1st third: < 166.1 cm, 2nd third: 166.1-172.0 cm, 3rd third: > 172.0 cm for males.

‡‡ n=111 due to missing data.

§§ n=58 due to missing data.

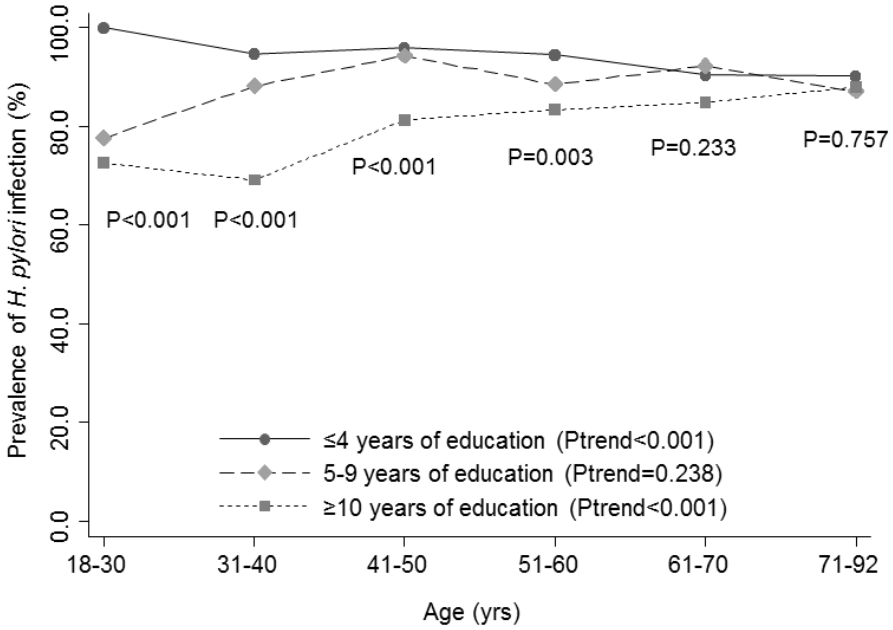
Figure 1. Flowchart describing the participants included in the analyses.



* The participants' infection status was classified as negative if the antibody concentration was lower than 16 RU/ml, borderline if the antibody concentration was equal or higher than 16 RU/ml and lower than 22 RU/ml, and positive if the antibody concentration was 22 RU/ml or higher. For analysis, subjects having borderline results were considered infected.

† CagA seropositivity was evaluated following the criteria recommended by the manufacturer: presence of the 116kD band (CagA) with one or more of the following bands: 89kD (VacA); 37kD; 35kD; 30kD (UreA) and 19.5kD together.

Figure 2. Prevalence of *Helicobacter pylori* infection according to age and education.



P refers to the comparisons of the prevalence of *H. pylori* infection between different levels of education, within each age group.

P for trend refers to the pattern of the prevalence of *H. pylori* infection across age groups, in each education strata

PAPER II

Prevalence, incidence and risk factors for *Helicobacter pylori* infection in a cohort of Portuguese adolescents (EpiTeen).

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Alimentary Tract

Prevalence, incidence and risk factors for *Helicobacter pylori* infection in a cohort of Portuguese adolescents (EpiTeen)

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ABSTRACT

Background: *Helicobacter pylori* infection is acquired mainly during childhood, but it may occur throughout life. Understanding the determinants of infection at different ages is essential to clarify dynamics of *H. pylori* related diseases and to design preventive strategies.
Aim: To estimate the prevalence of *H. pylori* infection at the age of 13 and the incidence after a 3-year follow-up and to identify risk factors for infection.
Subjects and methods: Adolescents born in 1990 were recruited in schools from Porto. Whole-cell anti-*H. pylori* IgG antibodies were quantified by ELISA. Prevalence ratios (PR) and incidence rate ratios (RR) adjusted for parental education were computed at baseline ($n = 1312$) and at follow-up ($n = 280$).
Results: The prevalence was 66.2%, lower in subjects with more educated parents (PR = 0.72, 95%CI: 0.63–0.82), and higher for those having more than one sibling (PR = 1.10, 95%CI: 1.02–1.19) and for smokers (PR = 1.11, 95%CI: 1.02–1.20). The incidence was 4.1/100 person-years. Smoking (RR = 2.35, 95%CI: 1.16–4.75) and type of school (RR = 0.38, 95%CI: 0.16–0.95) were associated with the incidence of infection.
Conclusions: Prevalence and incidence of *H. pylori* infection were high, suggesting that gastric cancer will remain an important public health problem in this generation of Portuguese. We identified smoking as a modifiable risk factor for infection.

1. Introduction

Half of the world adult population is infected by *Helicobacter pylori* [1], a human carcinogen [2] that is acquired mainly in childhood [3]. The prevalence varies appreciably across geographical areas [4,5] and is inversely associated with socioeconomic status or household hygiene and sanitation [6]. Over the last decades, the prevalence of infection in adult populations declined to below 50% in the more affluent countries of Europe [7–9], and this has contributed to the steep decline in gastric cancer incidence and mortality [10–12]. Despite the steady declines observed in Portugal since the 1970s [10,12,13], the country presented the highest gastric cancer incidence (19.2 per 100 000 in males and 9.2 per 100 000 in females) and mortality rates (15.0 per 100 000 men, and 6.8 per 100 000 women) in the European Union (27 countries) in 2008 [14].

Age at acquisition of infection is an important determinant of the *H. pylori*-related gastric outcomes [15]. The birth-cohort pattern observed for gastric and duodenal ulcer could be explained by the decrease in the frequency of *H. pylori* infection accompanied by a simultaneous shift to acquisition at older ages [15]. High prevalence of infection at younger ages is associated with higher gastric cancer mortality than in settings with a similar frequency of infection in older subjects [16] but later acquisition.

Information on the incidence and prevalence of *H. pylori* infection and the understanding of its determinants at different ages may contribute to better understand the relation between this infection and the frequency of gastric cancer, ultimately supporting the definition of a life course framework for gastric pathology research and allowing the estimation of the future burden of gastric disease. Therefore, we aimed to quantify the prevalence of *H. pylori*

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infection at the age of 13 and the incidence after a 3-year follow-up in a cohort of Portuguese adolescents, and to identify risk factors for infection at different ages.

2. Methods

A sample of adolescents born in 1990 was recruited in the schools from Porto, Portugal, in 2003/2004 (the EpiTeen cohort). School is compulsory at this age, and virtually all children are registered in a school of their dwelling area. A detailed description of the general selection procedures and participants' characteristics was published before [17,18]. In brief, the executive boards of all schools from Porto (27 public and 24 private) allowed the research team to contact the eligible students and their families. In compliant schools [all the public and 19 (79%) of the private], 2787 eligible adolescents were identified (2126 in public and 661 in private schools). We classified as refusals the eligible subjects who did not return the signed informed consent form (n = 583, 20.9%). Those agreeing to participate were contacted at school: 44 students (1.6%) could not be reached during the evaluation period because were missing classes; the remaining 2160 (1651 public and 509 private school students) provided information at least for part of the planned assessment (Fig. 1). This resulted in a proportion of participants of 77.5%, similar in public and private schools (77.7% *vs.* 76.7%, P = 0.613) [17].

During the baseline evaluation the participants answered two standardized questionnaires: one was completed by the adolescents at home, with the help of their legal guardians, to obtain data on social, demographic and behavioural characteristics as well as on individual and family history of disease; the other, used to collect information on health behaviours, particularly physical activity, smoking and alcohol consumption, was completed by the adolescents at school, during the visit of the research team. A physical examination was performed at school, between 8 a.m. and 10 a.m., by a team of experienced nurses, nutritionists and physicians, following standardized procedures. A 12-h overnight intravenous blood sample was obtained from 1390 participants. For the present study, we assessed the *H. pylori* status of 1312 subjects (94.4%) from which a large enough aliquot was available (Fig. 1).

During a follow-up conducted in 2007/2008, information regarding *H. pylori* infection was available for 280 (63.1%) participants without evidence of infection at the baseline evaluation (median follow-up: 37 months) (Fig. 1). A random sample, stratified by parental education, of approximately 10% of the participants with evidence of infection at baseline (n = 76) was used to quantify the seroreversion rate (median time of follow-up: 39 months).

Serum samples were kept frozen at −80 °C until analysis. Serum anti-*H. pylori* IgG titres were assessed by ELISA (Anti-*H. pylori* ELISA, EuroImmun, Lubeck, Germany). The participants' infection status was classified as negative if the antibody concentration was lower than 16 RU/ml, borderline if the antibody concentration was equal or higher than 16 RU/ml and lower than 22 RU/ml, and positive if the antibody concentration was 22 RU/ml or higher. For the current analysis, the adolescents with a borderline result (first evaluation: n = 22; second evaluation: n = 6) were considered infected.

2.1. Statistical analysis

For data analysis, parental education was defined as the highest educational level achieved by any one of the parents. The crowding index was calculated as the number of individuals living in the same house over the number of rooms; the cut point used to create a dichotomous variable was 1.3, which corresponds to the median in this population. The participants were classified as ever or never having tried smoking and alcohol consumption at the age of 13. The history of antibiotics use was also assessed in the follow-up evaluation.

Proportions were compared using the χ^2 test, or Fisher's exact test, when appropriate. Binomial regression was used to estimate parental education-adjusted prevalence ratios (PR) and the corresponding 95% confidence intervals (CI). Poisson regression was used to compute parental education-adjusted incidence rate ratios (RR) and the corresponding 95%CI. The statistical analysis was conducted using STATA®, version 9.2.

2.2. Ethics

The study was approved by the Ethics Committee of Hospital S. João and written consent was obtained from both legal guardians and adolescents.

3. Results

3.1. Prevalence of H. pylori infection at the age of 13

At baseline, the prevalence of *H. pylori* infection was 66.2% (95%CI: 63.6–68.7%).

As shown in Table 1, there was an inverse association between infection and the mother's and father's education. The adolescents studying in private schools were less likely to be infected (private *vs.* public: PR = 0.86, 95%CI: 0.78–0.96). The prevalence of infection was higher in subjects with more siblings (>1 *vs.* ≤1: PR = 1.12, 95%CI: 1.04–1.22), living in houses with a higher crowding index (>1.3 *vs.* ≤1.3: PR = 1.09, 95%CI: 1.01–1.19) or smokers (ever *vs.* never: PR = 1.12, 95%CI: 1.03–1.22). No significant associations were found with gender, bathing habits or care in washing hands before handling food (Table 1).

After adjustment for parental education, the number of siblings (>1 *vs.* ≤1: PR = 1.10, 95%CI: 1.02–1.19) and tobacco consumption (ever *vs.* never: PR = 1.11, 95%CI: 1.02–1.20) remained statistically significant (Table 1).

3.2. Incidence of H. pylori infection between the ages of 13 and 17

The incidence rate was 4.1/100 person-years (95%CI: 3.0–5.8).

A strong positive association was found between smoking at the age of 13 and incidence of *H. pylori* infection (ever *vs.* never: RR = 2.34, 95%CI: 1.16–4.72), even after adjustment for parental education (ever *vs.* never: RR = 2.35, 95%CI: 1.16–4.75). Studying in a public school was also a risk factor for the acquisition of *H. pylori* infection, independently from parental education, (private *vs.* public: RR = 0.38, 95%CI: 0.16–0.95) (Table 2).

The seroreversion rate was 1.6/100 person-years (95%CI: 0.6–4.4).

4. Discussion

The present study allowed us to estimate the point prevalence and the incidence of *H. pylori* infection in a representative sample of urban adolescents. The main findings were that the incidence of *H. pylori* infection was high throughout adolescence, despite the already high prevalence observed at the baseline age of 13. Social class, represented by parental education, was a relevant determinant of the prevalence at baseline, and smoking was associated with the incidence of infection during adolescence.

The prevalence observed in this study was higher than reported for most European countries, and even many South American, African and Asian settings [19–22], where it ranged from less than 10% to 64%. The prevalence of infection was 69% in Vietnam, in adolescents aged between 10 and 14 years evaluated in 2005 [23], 78%

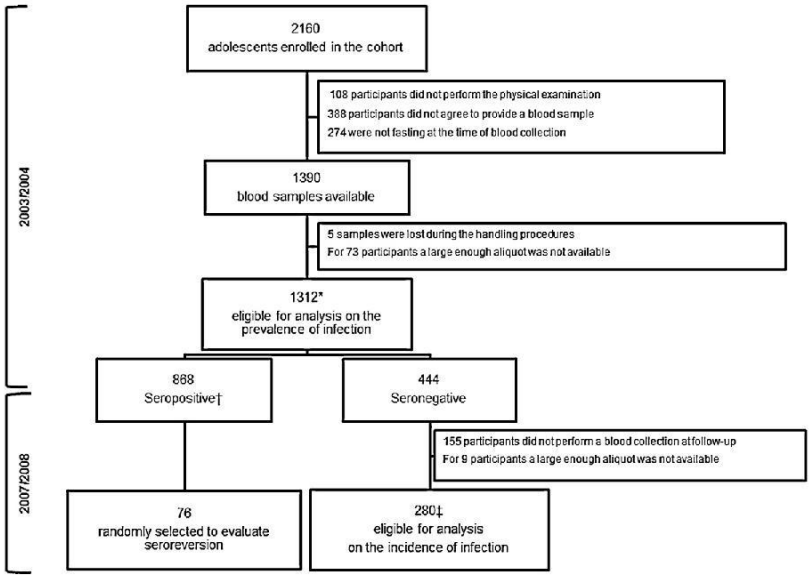

Fig. 1. Flow-chart describing the participants included in the analyses. *Participants without *H. pylori* testing had more educated parents (highest educational level achieved by any one of the parents – median number of school years: 11.0 *vs.* 10.0, $P = 0.052$), but a similar sex distribution (males: 49.8% *vs.* 47.3%, $P = 0.258$). †The participants' infection status was classified as negative if the antibody concentration was lower than 16 RU/ml, borderline if the antibody concentration was equal or higher than 16 RU/ml and lower than 22 RU/ml, and positive if the was 22 RU/ml or higher. ‡Participants with no information on *H. pylori* infection status at follow-up had less educated parents (median number of school years: 11.0 *vs.* 12.0, $P = 0.032$), but there were no significant differences regarding gender (males: 52.4% *vs.* 50.0%, $P = 0.620$).

in Rondônia, Brazil, in a sample of participants aged 11–20 years, evaluated in 2010 [24], and 82% in Iran, in subjects aged below 15 years, evaluated in 2006 [25]. In Portugal, the first national study addressing *H. pylori* prevalence (assessed by serology), published in 1994, showed that the prevalence of infection among children aged between 3 and 14 years was 46.2% [26]. In a recent study conducted in the south of Portugal, the prevalence of *H. pylori* infection (assessed by stool antigen test) in children aged between 3 and 14 years of age was 39.3%, and 51.5% in children aged between 11 and 15 years [27]. The prevalence found in our study is higher, which explains the higher gastric cancer incidence found in the North of Portugal [13,28].

Direct comparisons between studies assessing the frequency of *H. pylori* infection in adolescents is hampered by the diversity of age ranges, duration of follow-up, types of populations and characteristics of the diagnostic tools. The annual rates of seroconversion ranged from 0.1% to 1.9% in adolescents from high income countries, as assessed in the late 1990s [19]. In more recent studies, the annual rates were 1.5% in Japan [29], 1.4% in the USA [30], 2.5% in Turkey [31] and 7.0% in Turkish children living in Germany [31,32]. In a study from the South of Portugal, conducted between 2003 and 2006 it was 10.5/100 child-years in the age group 11–15 years [27], though only 52% participated in the follow-up study and from these 60% were lost during the 3-year follow-up.

A poor socio-economic condition favours infection, likely through poor sanitation, deficient hygiene and overcrowding [33–35]. We used education of the parents and type of school attended (private or public) as a surrogate measure of socio-economic status, and confirmed its importance as a determinant of infection. When including other potential markers of socioeconomic status in the model, such as smoking and alcohol consumption, the results remained essentially unchanged (data not shown). The number of siblings and household overcrowding during childhood reflect the probability of close contacts with infected individuals and has also been associated with *H. pylori* infection [30,36–38]. Our results confirm the role of the number of siblings for the occurrence of infection in the childhood but not in adolescence. However, the interpretation of this result is limited by the fact that we were not able to measure *H. pylori* infection in the household members. The crowding index was based on the information collected at 13 years and it may not totally reflect the crowding conditions during early childhood, which may have contributed to the lack of association between this exposure and infection at the baseline; on the other hand, it is a measure of the crowding conditions in adolescence, and the lack of its association with the acquisition of infection is likely to reflect the more important effect of other factors than family close contacts for the acquisition of the infection at these ages.

We also evaluated bathing and hand-washing habits and hygiene in handling foods, but we found no associations with infection in these adolescents. A previous analysis of this dataset showed that the lowest hand washing frequency was associated with higher probability of diarrhoea occurrence [39], and therefore it is unlikely that the lack of association observed in the present investigation reflects underestimation due to social desirability bias [40].

Although controversial, there is a body of evidence regarding the relation between smoking and *H. pylori* infection [6]. The fact that tobacco smoking is associated with other lifestyles and with nutritional status [41–44] may contribute to the conflicting

Table 1
Factors associated with the prevalence of *H. pylori* infection at the age of 13.

	N	*H. pylori*-infected, *n* (%)	Crude PR (95%CI)	Adjusted[a] PR (95%CI)
Mother's education[b]				
≤4 years	311	227 (72.3)	1.00	–
5–9 years	406	286 (70.4)	0.96 (0.88–1.06)	–
10–12 years	282	164 (58.2)	0.80 (0.71–0.90)	–
>12 years	254	141 (55.5)	0.76 (0.67–0.87)	–
Father's education[b]				
≤4 years	289	210 (72.7)	1.00	–
5–9 years	408	273 (66.9)	0.92 (0.83–1.02)	–
10–12 years	291	181 (62.2)	0.86 (0.76–0.96)	–
>12 years	215	112 (52.1)	0.72 (0.62–0.83)	–
Parental education[b]				
≤4 years	189	144 (76.2)	1.00	
5–9 years	438	311 (71.0)	0.93 (0.84–1.03)	
10–12 years	326	200 (61.4)	0.80 (0.72–0.90)	–
>12 years	310	170 (54.8)	0.72 (0.63–0.82)	–
Type of school				
Public	996	681 (68.4)	1.00	1.00
Private	316	187 (59.2)	0.86 (0.78–0.96)	0.93 (0.83–1.03)
Gender				
Females	691	473 (68.4)	1.00	1.00
Males	621	395 (63.6)	0.93 (0.86–1.00)	0.92 (0.85–1.00)
Number of siblings[b]				
≤1	892	564 (63.2)	1.00	1.00
>1	401	285 (71.1)	1.12 (1.04–1.22)	1.10 (1.02–1.19)
Crowding index[b]				
≤1.3	392	242 (61.7)	1.00	1.00
>1.3	872	587 (67.3)	1.09 (1.01–1.19)	1.05 (0.96–1.15)
Smoking[b]				
Never tried	958	612 (63.9)	1.00	1.00
Ever tried	308	220 (71.4)	1.12 (1.03–1.22)	1.11 (1.02–1.20)
Alcohol drinking[b]				
Never tried	575	390 (67.8)	1.00	1.00
Ever tried	688	438 (63.7)	0.94 (0.87–1.02)	0.96 (0.89–1.04)
Bath habits[b]				
Bath ≥4 times/week	1158	755 (65.2)	1.00	1.00
Bath <4 times/week	132	92 (69.7)	1.07 (0.95–1.21)	1.06 (0.94–1.19)
Washing hands before handling food[b]				
Always	913	604 (66.2)	1.00	1.00
Not always	379	244 (64.4)	0.97 (0.89–1.06)	1.02 (0.93–1.11)

PR, prevalence ratio; 95%CI, 95% confidence interval.
[a] Adjusted for parental education (highest educational level achieved by any one of the parents).
[b] The sum of participants in each class is lower than the total (n = 1312) due to missing information.

findings on this topic. In our study, we opted for providing the PR and RR estimates adjusted only for parental education, because the point estimates did not vary meaningfully when BMI (above the 95th percentile, between the 85th and the 95th percentile and below the 85th percentile, as recommended for children and adolescents according to the age- and sex-specific BMI reference percentiles developed by the United States Centers for Disease Control and Prevention [45,46]) or dietary patterns were included in the model. This may reflect the fact that parental education is a surrogate for socioeconomic status and lifestyle exposures, but also the lack of association between each of the latter and *H. pylori* infection. In our study, after adjustment for parental education we did not find significant associations between BMI and *H. pylori* infection, (above the 95th percentile *vs.* below the 85th percentile: PR = 0.92, 95%CI: 0.80–1.06; between the 85th and the 95th percentile *vs.* below the 85th percentile: PR = 0.92, 95%CI: 0.75–1.12) or between dietary patterns (unpublished results) and infection ("High calorie" *vs.* "Healthy": PR = 1.01, 95%CI: 0.84–1.22; "Low calorie" *vs.* "Healthy"; PR = 1.08, 95%CI: 0.93–1.25; "Undifferentiated" *vs.* "Healthy": PR = 0.96, 95%CI: 0.81–1.13). When including smoking habits in these models, the point estimates remained essentially unchanged. Although the association observed in our study between tobacco use and infection is relatively weak, it is biologically plausible and smoking was also shown to contribute for the failure of *H. pylori* eradication [47]. Our results show that there is potential for intervention in the reduction of *H. pylori* infection during adolescence and this should be taken into account when estimating the potential benefits of tobacco control measures at early ages. A greater frequency of *H. pylori* infection in smokers may also at least in part contribute to explain the relation between tobacco and gastric cancer [12,48]. On the other hand, the lack of association between alcohol consumption and infection is in accordance with previous reports [6].

Our results show that nearly 16 in each 1000 *H. pylori* infected adolescents undergo seroreversion each year. In one of these adolescents a short course of antibiotics for respiratory disease had been prescribed a few months before the follow-up assessment, but no history of use of antibiotics was reported in the same period for the remaining. Serologic tests in children are not as accurate as in adults [49]; although using the cut-off values defined for adults may yield a lower sensitivity in infants, after the age of 12 years sensitivity and specificity become as high as in adults [49]. According to the manufacturer's description of the product, the test used to assess infection status in the present investigation has a sensitivity of 100% and a specificity of 94%, in adults. We classified the participants with borderline serum IgG titres as infected, but the results were not meaningfully different when participants with borderline results were considered not infected (data not shown). Furthermore, the declines in the antibody concentration of the participants in whom seroreversion was observed ranged between 72%

Table 2
Factors associated with the incidence of *H. pylori* infection after the age of 13.

	N	Incident cases of infection	Person-years at risk	Crude RR (95%CI)	Adjusted[a] RR (95%CI)
Mother's education					
≤4 years	48	7	144	1.00	–
5–9 years	75	9	233	0.79 (0.30–2.13)	–
10–12 years	71	10	213	0.96 (0.37–2.54)	–
>12 years	86	9	256	0.72 (0.27– 1.94)	–
Father's education[b]					
≤4 years	52	8	158	1.00	–
5–9 years	81	11	246	0.88 (0.35–2.20)	–
10–12 years	66	6	202	0.59 (0.20–1.69)	–
>12 years	77	10	226	0.87 (0.34–2.21)	–
Parental education					
≤4 years	31	5	92	1.00	–
5–9 years	74	8	232	0.63 (0.21–1.94)	
10–12 years	70	9	213	0.78 (0.26–2.32)	
>12 years	105	13	309	0.77 (0.28–2.17)	–
Type of school					
Public	190	29	570	1.00	1.00
Private	90	6	276	0.43 (0.18–1.03)	0.38 (0.16–0.95)
Gender					
Females	140	15	431	1.00	1.00
Males	140	20	415	1.38 (0.71–2.70)	1.39 (0.71–2.71)
Number of siblings					
≤1	210	23	635	1.00	1.00
>1	70	12	211	1.57 (0.78–3.16)	1.58 (0.78–3.17)
Crowding index[b]					
≤1.3	98	13	292	1.00	1.00
>1.3	178	21	542	0.87 (0.44–1.74)	0.88 (0.44–1.75)
Smoking[b]					
Never tried	218	22	664	1.00	1.00
Ever tried	53	12	155	2.34 (1.16–4.72)	2.35 (1.16–4.75)
Alcohol drinking[b]					
Never tried	112	16	341	1.00	1.00
Ever tried	164	19	492	0.82 (0.42–1.60)	0.82 (0.42–1.60)
Bath habits[b]					
Bath ≥4 times/week	250	30	757	1.00	1.00
Bath <4 times/week	29	5	86	1.47 (0.57–3.78)	1.47 (0.57–3.78)
Washing hands before handling food					
Always	186	24	569	1.00	1.00
Not always	94	11	277	0.94 (0.46–1.92)	0.94 (0.46–1.92)

RR, incidence rate ratio; 95%CI, 95% confidence interval.
[a] Adjusted for parental education (highest educational level achieved by any one of the parents).
[b] The sum of participants in each class is lower than the total (n = 280) due to missing information.

and 99%, supporting the hypothesis of a previous infection that was resolved.

Two patterns of *H. pylori* prevalence in respect to age have been described. In middle and low income countries infection is acquired mainly in childhood and may reach nearly 100% during adulthood. In high income countries, the infection is less common in young children and gradually increases with age [5]. Portugal presents a very high prevalence of infection in adolescence, similarly to what is observed in middle and low income settings, but also a high incidence between the ages of 13 and 17, showing that the acquisition in this period is not negligible and contributes for the high prevalence later in life.

In conclusion, approximately two-thirds of these Portuguese 13 year-old were infected with *H. pylori*, and the incidence of infection remained high during the adolescence. Our results suggest that stomach cancer incidence and mortality in Portugal are likely to remain among the highest in Europe.

Conflict of interest statement
The authors declare that there are no conflicts of interest to disclose.

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PAPER III

The role of child-care attendance in the early life prevalence of *Helicobacter pylori* infection: results from the Portuguese birth cohort Geração XXI.

Abstract

Child-care attendance and *Helicobacter pylori* infection in the Portuguese birth cohort Geração XXI

Background: *Helicobacter pylori* infection is acquired predominantly during childhood. However, the role of child-care attendance as an independent determinant remains to be understood.

Aims: To quantify the association between child-care attendance and *H. pylori* infection in early life.

Methods: The study was nested within Geração XXI, a birth cohort assembled in Portugal. Serum anti-*H. pylori* IgG was quantified by ELISA in 1047 children between their 4^{th} and 5^{th} birthday, and information on child-care attendance since birth was collected. Odds ratios (OR) and 95% confidence intervals (95%CI), adjusted for the children's age and number of siblings, as well as, maternal education and infection status were computed using unconditional logistic regression.

Results: The prevalence of *H. pylori* infection was 30.6% (95CI%: 27.9-33.6), and it increased significantly with the cumulative time of attendance of day-care centers/homes (from 13.2% among never attendees to 40.2% among those attending >36 months, P for trend <0.001). The OR was 4.88 (95%CI: 2.55-9.35) among those attending these institutions for more than three years, in comparison with never attendees.

Conclusion: *H. pylori* infection remains a frequent and early event in Portugal. Day-care attendance increases the risk of infection, making this setting a target for preventive actions.

Introduction

More than half of the world adult population is infected with *Helicobacter pylori*. The higher prevalences are observed in the less affluent regions and in population groups with lower socioeconomic status [1]. Although the infection may be acquired throughout life, incidence rates tend to be higher among children, suggesting that infection occurs predominantly in the first years of life, especially in developing countries [1].

The transmission of *H. pylori* is still not entirely understood, but human-to-human spread through oral-oral or fecal-oral route is the most likely mechanism [2]. The contacts between children and their mothers and between siblings have been described as the main factors contributing to the transmission of infection in early life [3-5]. However, the role of child-care attendance as a risk factor for infection remains to be understood.

A growing number of children are cared out of home and are exposed to the interaction with a high number of young children, sometimes in the absence of appropriate hygienic practices, which favors the transmission of pathogenic agents [6]. A large body of evidence, mostly gathered in the USA, and to a lesser extent in Europe, underlies that children attending child-care centers are at higher risk of diarrhea [6,7] especially under the age of three. Group-care was also shown to increase the risk of acute diarrhea whatsoever the specific setting [8]. Given the expected role of the fecal-oral route of transmission, and the importance of the exposure to *H. pylori* in early life, group-care is likely to be an important determinant of the infection. A recent study showed that *H. pylori* DNA was detected in 55% of the toys collected in a day-care center [9], and a higher risk of infection among children attending child-care institutions has been reported [10]. However, the effect of the age at onset of attendance, number of hours in day-care centers or the number of children taken care together has seldom been addressed [11,12]. Understanding and quantifying such effect may translate into preventive action.

We aimed to quantify the prevalence of *H. pylori* infection in 4 to 5 year old children, and to assess the independent effect of child-care attendance in the early life acquisition of the infection.

Materials and Methods

This study was conducted in a subsample of the birth cohort Geração XXI. As previously described [13], the cohort recruited 8647 newborns at all public maternity units covering the metropolitan area of Porto, Portugal, between April 2005 and August 2006. At baseline, 91.4% of the invited mothers accepted to participate and information was collected during hospital stay through a face-to-face interview conducted within 72 hours after delivery, by trained interviewers using structured questionnaires. Venous blood samples from both parents were also collected at baseline and serum was stored at -80ºC until analysis.

The cohort was re-evaluated 4 to 5 years after the baseline assessment, mainly by personal interviews using structured questionnaires. Venous blood samples from children were collected and serum was stored at -80ºC until analysis.

Whole-cell IgG antibodies against *H. pylori* were quantified by Enzyme-Linked Immunoabsorbent Assay (ELISA) (EuroImmun®, Germany). Regarding the *H. pylori* infection status of each participant, results were considered negative when the IgG concentrations were below 16 RU/ml, borderline for 16-22 RU/ml, and positive for infection when the titers were ≥22 RU/ml. For analysis, subjects with borderline IgG titres were classified as infected.

Information was collected on the different child-care options since birth, and on the age at beginning and end of care in each setting, when applicable. Child-care could be provided by parents or other family members in their houses, in day-care homes (care provided by a non-family member at her/his house) or day-care centers (group-care provided in institutions).

The child-care settings that were currently being attended by the children were also characterized regarding the number of hours per week spent in each setting and the number of children that were cared together by the same provider(s).

Children whose parents had provided a blood sample at the baseline evaluation were eligible for this study (n=1959). Mothers from whom a blood sample was collected at baseline were more educated than the remaining (median number of complete education years: 11 vs. 9 years, P<0.001), but no significant differences were found regarding their age (median age: 30 vs. 30 years, P=0.933).

From the eligible children, 1740 were re-evaluated. Their mothers were significantly older and more educated than those of the children lost during follow-up (median age: 30 vs. 27 years, P<0.001 and median number of complete education years: 11 vs. 9 years, P<0.001). A blood sample could not be collected from 693 children that participated in the follow-up, as 344 were evaluated by telephone or post

interview, for 222 the blood collection could not be performed due to technical difficulties and 127 refused to provide a blood sample. The mothers of these children were significantly more educated than those of children providing a blood sample (38.2% *vs.* 28.6% with more than 12 years of education, P<0.001).

The present analyses include therefore 1047 children (median age: 4.2 years), 1036 mothers (median age: 34 years; median number of complete education years: 12) and 409 fathers (median age: 36 years; median number of complete education years: 9) for whom the characterization of the *H. pylori* infection status was accomplished.

The independent relation between the attendance of group-care since birth and the *H. pylori* infection status of the children was quantified through odds ratios (OR), and the corresponding 95% confidence intervals (95%CI), computed using non-conditional logistic regression including terms for children's age, number of siblings, parental education and infection status, and attendance of different types of day-care, as applicable. All analyses were conducted using STATA® (College Station, TX, 2005), version 11.2.

The study protocol was approved by Ethics Committee of the Hospital de S. João, and written informed consent was obtained from the parents.

Results

The overall prevalence of *H. pylori* infection was 30.6% (95CI%: 27.9-33.6) among the children, (31.4%, 95%CI: 27.4-35.7 in girls and 29.9%, 95%CI: 26.1-34.0 in boys), 73.7% (IC95%: 71.0-76.4) among the mothers and 79.5% (95%CI: 75.2-83.3) among the fathers.

The prevalence was significantly higher in children over age 4.2 years (the median age in this sample) compared with those aged ≤4.2 years (35.0%, 95CI%: 30.2-40.1 vs. 28.2%, 95CI%: 24.8-31.8; P=0.022). Infection was less frequent in children of more educated mothers (higher vs. basic education: 23.4% vs. 33.6%; P for trend=0.002) (Table 1).

The prevalence of infection was higher among children whose mother or father were infected, though only the former association was significant. When considering simultaneously the infection status of both parents, the strongest associations were observed when only the mother (adjusted OR=2.66, 95%CI: 0.76-9.27) or both the mother and the father were infected (adjusted OR=3.27, 95%CI: 1.09-9.83), in comparison with children with both parents testing negative for infection (Table 2).

Only 8.9% of the children did not attend child-care settings outside their families and 2.3% attended exclusively day-care homes since birth, while most of them (74.9%) attended only day-care centers. The remaining 13.9% had been cared in both day-care centers and day-care homes. An increased risk of infection was observed in the children who attended either day-care centers (OR=1.97, 95%CI: 1.21-3.21) or day-care homes (OR=1.46, 95%CI: 1.02-2.08). The risk of infection increased significantly (P for trend <0.001) with the cumulative time of attendance of day-care centers or homes. The OR was 1.12 (95%CI: 0.44-2.80) for children cared in these settings for 1 to 6 months, and increased to 4.88 (95%CI: 2.55-9.35) among those attending these child-care settings for more than three years, in comparison with children cared for within their families during the whole period (Table 3).

Among the children who were attending a child-care center, no independent relation was observed between the size of the group of children cared together or the time spent in day-care centers (Table 4).

Discussion

This study shows that nearly one-third of the 4 year old children in Porto are infected by *H. pylori*, and that the risk of infection increases significantly with the cumulative time of attendance of day-care centers or homes since birth.

This study provides robust evidence for measures to be implemented in child-care settings for the prevention of *H. pylori* infection in early childhood. However, some limitations need to be discussed.

In a recent review on the accuracy of *H. pylori* diagnostic tests in children [14], five articles studying children under 6 years of age and measuring IgG by enzyme immunoassay, showed that these tests may have a lower sensitivity in children than in adult populations, although the specificity was similar or even better in children. According to the manufacturer's description [15], the ELISA test used in our study had a sensitivity of 100% and specificity of 94% in 70 clinically characterized patient samples, but the accuracy of the test was not assessed in children; however, in a sample of 500 healthy blood donors the prevalence of infection was 25%, while in 88 children aged below 10 years the prevalence of positive results was 9%. The prevalence of infection in the children evaluated in our study was high (30.6%), in broad agreement with the prevalences in adolescents (≈66%) and adults from the same population (≈75-90%) [16,17]. This underlies that misclassification of infected children as non-infected (likely to be non-differential) is unlikely to have occurred to an extent that compromises our conclusions, in particular since group day-care was strongly associated with infection. Furthermore, the relation with other determinants of infection is in accordance with the previous evidence, namely regarding the relation with markers of socioeconomic status [1,18], breastfeeding [19], or use of antibiotics [20,21]. There were non-significant trends towards a decrease in the prevalence of infection with younger age of the mothers and the fathers, which were attenuated after adjustment for children's age, and parental education (data not shown). The similar prevalences of *H. pylori* infection obtained for children from mothers with different nationalities may reflect that these were predominantly from settings where the prevalence of infection in the adult population is as high as the observed in Portugal, namely Brazil, African countries and Eastern European countries [22]. We also conducted a sensitivity analysis considering the participants with borderline results as uninfected, which resulted in a still high prevalence of infection of 25.8% (95%CI: 23.2-28.6) and the associations with the exposures evaluated remained essentially the same.

The high prevalence of infection is in accordance with the high age-specific estimates obtained in previous studies that evaluated adolescent and adult city

dwellers from Porto [16]. Further, a recently published report on an investigation conducted a decade ago in Lisbon [23], assessing infection using a stool antigen test, yielded prevalences of infection of 19.9% and 37.0% in children aged 0-5 years and 6-10 years, respectively.

Other previous reports [23,24] showed odds ratios of similar magnitude for the attendance of child-care institutions. Our investigation, however, adds to the existing evidence the demonstration of a strong dose-effect relation between the duration of child-care attendance and infection. It is impossible to disentangle the effect of the cumulative exposure from that of starting to be cared in group at earlier ages. We may hypothesize, however, that in this specific setting the effect of the cumulative exposure was more important because high rates of infection are observed throughout life and not only among young children [16].

The lack of association between the size of the groups in day-care or the weekly duration of stay in these institutions may reflect the fact that the long-term exposure to group-care may overcome the potential effect of these factors. However, these results are limited by the fact that the analyses only took into account the characteristics of the setting where children are currently being cared, which do not necessarily reflect these markers of intensity of exposure in younger ages. The small number of children attending only day-care homes precluded the evaluation of the specific effect of this type of care on the prevalence of infection.

We showed a strong relation between the infection status of the parents and their children, especially when both parents were infected. Even if the serological evidence of infection does not necessarily correspond to current infection, especially among adults, this variable is a surrogate of the potential effects and particularly for mother-to-child transmission of the infection. This allowed a finer adjustment for these potential confounders and further strengthens our conclusions. Despite data on the infection status was available only for a subsample of the fathers, we conducted additional analyses including only these participants and further accounting for the potential confounding effect of the infection status of the fathers (data not shown); the conclusions remained unchanged.

The mothers of the children whose data were considered for these analyzes were more educated than those excluded. Although this has contributed for an underestimation of the prevalence of infection, our conclusions on the association between child-care attendance and infection are based in strong and consistent OR estimates, and are unlikely to be influenced by this selection bias.

H. pylori infection is the major risk factor for gastric cancer and the possibility of it being considered a necessary cause for the occurrence of this outcome is currently

under discussion [25]. If this hypothesis is confirmed, the interventions for primary prevention of the infection may be sufficient to prevent gastric cancer. However, some of the gastric precancerous lesions that are relatively frequent in populations with a high frequency of infection may be irreversible [26,27], and therefore the strategies for prevention of the infection in early life are more likely to yield an effective control of gastric cancer. The results from this study identify day-care centers and day-care homes as targets for interventions aiming the prevention of the infection during childhood.

In conclusion, our results show that the prevalence of infection among children is high in Portugal. Day-care attendance increases the risk of infection, making this setting a potential target for preventive actions.

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Table 1. *Helicobacter pylori* infection among the children, according to socio-demographic factors, breastfeeding and parental characteristics.

	H. pylori infection status *		
	Negative n (%) †	Positive n (%) †	P
Age (years)			
≤4.2 ‡	481 (71.8)	189 (28.2)	0.022
>4.2	245 (65.0)	132 (35.0)	
Sex			
Female	349 (68.6)	160 (31.4)	0.597
Male	377 (70.1)	161 (29.9)	
Number of siblings			
0	328 (68.0)	154 (32.0)	0.325
1	338 (72.1)	131 (27.9)	
>1	60 (63.2)	35 (36.8)	
Crowding index§			
<1	250 (72.7)	94 (27.3)	0.258
1	250 (68.7)	114 (31.3)	
1.1-1.5	195 (68.4)	90 (31.6)	
>1.5	30 (60.0)	20 (40.0)	
Mother's age (years) ¶			
<30	172 (65.2)	92 (34.8)	0.162
30-40	471 (71.5)	188 (28.5)	
>40	73 (70.9)	30 (29.1)	
Mother's education (years) ¶			
0-8	138 (66.4)	70 (33.6)	0.007
9	148 (63.5)	85 (36.5)	
10-12	202 (70.6)	84 (29.4)	
>12	226 (76.6)	69 (23.4)	
Mother's country of birth ¶			
Portugal	644 (70.1)	275 (29.9)	0.930
African countries	29 (67.4)	14 (32.6)	
South-American countries	17 (70.8)	7 (29.2)	
Other European countries	25 (65.8)	13 (34.2)	
Father's age¥			
<30	82 (67.8)	39 (32.2)	0.283
30-40	420 (70.8)	173 (29.2)	
>40	134 (77.0)	40 (23.0)	
Father's education (years) ¥			
0-8	188 (69.4)	83 (30.6)	0.322
9	126 (71.6)	50 (28.4)	
10-12	167 (70.2)	71 (29.8)	
>12	149 (76.8)	45 (23.2)	
Exclusive breastfeeding (weeks)			
<9	198 (68.5)	91 (31.5)	0.487
9-16	254 (69.0)	114 (31.0)	
>16	190 (72.8)	71 (27.2)	
Child antibiotics' use (previous year)			
No	287 (66.3)	146 (33.7)	0.077
Yes	437 (71.4)	175 (28.6)	

* IgG concentrations <16 RU/ml (negative) or ≥16 RU/ml (positive); † the sum of the number of participants may be lower than the total due to missing data, and percentages may add up more than 100% due to rounding; ‡ median age in this sample; § number of persons per room (including bedrooms and living rooms); ¶ results are provided only for the children who live with their mothers (n=1026);¥ results are provided only for the children who live with their fathers (n=890).

Table 2. *Helicobacter pylori* infection among the children, according to the parental infection status.

Parental *H. pylori* infection status *	*H. pylori* infection status of the children * Negative n (%)†	Positive n (%)†	Crude OR (95%CI)	Adjusted‡ OR (95%CI)
Mothers §				
Negative	208 (76.8)	63 (23.2)	1 (reference)	1 (reference)
Positive	508 (67.1)	249 (32.9)	1.62 (1.18-2.23)	1.54 (1.11-2.13)
Fathers ¶				
Negative	64 (78.0)	18 (22.0)	1 (reference)	1 (reference)
Positive	223 (69.7)	97 (30.3)	1.55 (0.87-2.75)	1.41 (0.78-2.53)
Mothers and fathers ¥				
Both negative	28 (87.5)	4 (12.5)	1 (reference)	1 (reference)
Father positive and mother negative	63 (81.8)	14 (18.2)	1.56 (0.47-5.15)	1.55 (0.46-5.20)
Father negative and mother positive	34 (72.3)	13 (27.7)	2.68 (0.78-9.13)	2.66 (0.76-9.27)
Both positive	156 (66.4)	79 (33.6)	3.54 (1.20-10.46)	3.27 (1.09-9.83)

OR – odds ratio; 95%CI – 95% confidence interval

* IgG concentrations <16 RU/ml (negative) or ≥16 RU/ml (positive); † percentages may add up more than 100% due to rounding; ‡ adjusted for children's age (continuous), number of siblings (0, 1, >1) and maternal or/and paternal education (0-8, 9, 10-12, >12 years), as applicable; § data is available for 1028 mothers; ¶ data is available for 402 fathers; ¥ data is available for 391 mothers and fathers.

Table 3. *Helicobacter pylori* infection status in children, according to type of child-care attended since birth.

Attendance of:	*H. pylori* infection status * Negative n (%)†	Positive n (%)†	Crude OR‡ (95%CI)	Adjusted§ OR‡ (95%CI)
Day-care homes or centers				
None	79 (86.8)	12 (13.2)	1 (reference)	1 (reference)
Al least one type	629 (67.8)	299 (32.2)	3.13 (1.68-5.83)	3.25 (1.73-6.11)
Day-care centers				
No	92 (80.0)	23 (20.0)	1 (reference)	1 (reference)¶
Yes	621 (68.3)	288 (31.7)	1.86 (1.15-2.99)	1.97 (1.21-3.21)
Day-care centers (since age in months)				
Never	92 (80.0)	23 (20.0)	1	1
>36	271 (79.5)	70 (20.5)	1.03 (0.61-1.75)	1.09 (0.63-1.90)
25-36	132 (71.4)	53 (28.6)	1.61 (0.92-2.80)	1.88 (1.04-3.40)
13-24	62 (58.5)	44 (41.5)	2.84 (1.56-5.16)	3.22 (1.69-6.14)
6-12	47 (66.2)	24 (33.8)	2.04 (1.04-4.00)	2.22 (1.08-4.53)
0-6	108 (52.7)	97 (47.3)	3.59 (2.11-6.12)	4.70 (2.66-8.31)
Day-care centers (duration in months)				
Never	92 (80.0)	23 (20.0)	1 (reference)	1 (reference)¥
1-6	70 (76.9)	21 (23.1)	1.20 (0.62-2.34)	1.13 (0.56-2.27)
7-12	143 (80.3)	35 (19.7)	0.98 (0.54-1.76)	1.11 (0.60-2.04)
13-24	171 (73.1)	63 (26.9)	1.47 (0.86-2.53)	1.69 (0.95-3.01)
25-36	74 (64.9)	40 (35.1)	2.16 (1.19-3.93)	2.59 (1.37-4.90)
>36	161 (55.5)	129 (44.5)	3.20 (1.92-5.35)	4.12 (2.37-7.16)
Day-care homes				
No	606 (71.0)	247 (29.0)	1 (reference)	1 (reference)¶
Yes	102 (61.4)	64 (38.6)	1.54 (1.09-2.18)	1.46 (1.02-2.08)
Day-care homes (duration in months)				
Never	606 (71.0)	247 (29.0)	1 (reference)	1 (reference) ¥
1-12	18 (43.9)	23 (56.1)	3.13 (1.66-5.91)	2.79 (1.43-5.46)
13-36	57 (69.5)	25 (30.5)	1.08 (0.66-1.76)	1.33 (0.79-2.24)
>36	27 (62.8)	16 (37.2)	1.45 (0.77-2.74)	2.51 (1.26-5.02)
Day-care homes or centers				
None	79 (86.8)	12 (13.2)	1 (reference)	1 (reference)
Only day-care homes	13 (54.2)	11 (45.8)	5.57 (2.04-15.24)	5.67 (2.04-15.77)
Only day-care centers	527 (69.2)	235 (30.8)	2.94 (1.57-5.49)	3.09 (1.64-5.83)
Day-care homes and centers	89 (62.7)	53 (37.3)	3.92 (1.95-7.86)	3.76 (1.86-7.62)
Day-care homes or centers (duration in months)				
Never	79 (86.8)	12 (13.2)	1 (reference)	1 (reference)
1-6	57 (85.1)	10 (14.9)	1.15 (0.47-2.86)	1.12 (0.44-2.80)
7-12	120 (81.1)	28 (18.9)	1.54 (0.74-3.20)	1.62 (0.77-3.40)
13-24	138 (70.8)	57 (29.2)	2.72 (1.38-5.37)	2.89 (1.44-5.78)
25-36	79 (64.8)	43 (35.2)	3.58 (1.76-7.30)	3.78 (1.83-7.78)
>36	234 (59.2)	161 (40.8)	4.53 (2.39-8.59)	4.88 (2.55-9.35)

OR – odds ratio; 95%CI – 95% confidence interval

* IgG concentrations <16 RU/ml (negative) or ≥16 RU/ml (positive); †the sum of the number of participants may be lower than the total number of participants (1036 children for whom the infection status of the mother was also available) due to missing data, and percentages may add up more than 100% due to rounding; ‡ odds ratios computed for the comparison of positive/borderline vs. negative IgG concentrations; § adjusted for children's age (continuous), maternal education (0-8, 9, 10-12, >12 years), maternal infection status (negative, borderline/positive) and number of siblings (0, 1, >1); ¶ odds ratios further adjusted for the attendance of day-care homes or day-care centers, as applicable; ¥ odds ratios further adjusted for the duration of attendance of day-care homes (Never, 1-12, 13-36, >36 months) or day-care centers (Never, 1-6, 7-12, 13-24, 25-36, >36 months), as applicable.

Table 4. *Helicobacter pylori* infection status in children, according to the number of children in group-care and duration of stay in day-care centers, for children currently attending a day-care center (n=895).

Group-care and duration of stay in day-care centers:	*H. pylori* infection status * Negative n (%)†	Positive n (%)†	Crude OR‡ (95%CI)	Adjusted§ OR‡ (95%CI)
Number of children with the same carer(s)				
2-15	130 (67.7)	62 (32.3)	1 (reference)	1 (reference)
16-20	184 (66.7)	92 (33.3)	1.05 (0.71-1.55)	1.07 (0.71-1.62)
≥21	240 (71.4)	96 (28.6)	0.84 (0.57-1.23)	0.90 (0.60-1.35)
Duration of stay (hours/week)				
9-30	95 (74.8)	32 (25.2)	1 (reference)	1 (reference)
31-44	320 (70.6)	133 (29.4)	1.23 (0.79-1.93)	0.93 (0.58-1.50)
≥45	196 (62.6)	117 (37.4)	1.77 (1.12-2.81)	1.02 (0.61-1.70)

OR – odds ratio; 95%CI – 95% confidence interval

* IgG concentrations <16 RU/ml (negative), between 16 and 22 RU/ml (borderline) or ≥22 RU/ml (positive); †the sum of the number of participants may be lower than the total number of participants due to missing data, and percentages may add up more than 100% due to rounding; ‡ odds ratios computed for the comparison of positive vs. negative/borderline IgG concentrations; § adjusted for children's age (continuous), maternal education (0-8, 9, 10-12, >12 years), maternal infection status (negative, borderline/positive), number of siblings (0, 1, >1) and duration of attendance of day-care centers (Never, 1-6, 7-12, 13-24, 25-36, >36 months).

PAPER IV

Childcare attendance and *Helicobacter pylori* infection: systematic review and meta-analysis.

Childcare attendance and *Helicobacter pylori* infection: systematic review and meta-analysis

Joana Bastos[a,b,c], Helena Carreira[a,b], Carlo La Vecchia[d,e] and Nuno Lunet[a,b]

Helicobacter pylori infection is acquired predominantly during childhood. Childcare promotes interpersonal contact and may be an important determinant of infection. The aim was to quantify the association between childcare attendance and _H. pylori_ infection in childhood or adolescence. PubMed was searched up to July 2012 to identify eligible studies. The DerSimonian and Laird method was used to compute summary odds ratio (OR) estimates and 95% confidence intervals (CIs); heterogeneity was quantified with the I^2 statistic and explained through stratified analyses and metaregression. Sixteen studies compared participants attending childcare with those not exposed. The summary OR was 1.12 (95% CI: 0.82–1.52, I^2=77.4%). Summary estimates were similar for crude and adjusted estimates, and higher when the infection was evaluated in children of 3 years or younger (OR=2.00, 95% CI: 0.94–4.29, I^2=55.0%). Studies based on the detection of stool antigens yielded higher estimates (OR=2.65, 95% CI: 1.24–5.66, I^2=36.4%). Those conducted in settings with a high prevalence of _H. pylori_ infection yielded stronger associations (OR=1.44, 95% CI: 0.94–2.20, I^2=74.3%). In multivariate metaregression, there was no significant association with any of these variables; taking them into account contributed to a reduction of I^2 to 67%. The role of childcare as a risk factor for _H. pylori_ infection is confirmed by our results, especially in settings with a high prevalence of infection. However, the association was moderate, and the effect of the type of childcare setting or the duration or the intensity of exposure was seldom addressed, leaving considerable scope for improving our understanding of how this modifiable exposure contributes towards _H. pylori_ infection. _European Journal of Cancer Prevention_ 00:000–000 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Helicobacter pylori infection is the most important risk factor for gastric cancer (IARC, 1994). In the last few decades, the prevalence of infection in adult populations decreased to below 50% in the more affluent European countries (Gause-Nilsson *et al.*, 1998; Harvey *et al.*, 2002; Asfeldt *et al.*, 2008), and this has contributed towards the steep decrease in the incidence of gastric cancer and mortality (Levi *et al.*, 2004; Kamangar *et al.*, 2006). Worldwide, approximately one-third of the two million cases of cancer because of infections in 2008 were attributable to *H. pylori* (de Martel *et al.*, 2012). The prevalence of *H. pylori* varies considerably across geographical areas (Lunet and Barros, 2003; Lunet and Barros, 2006), and was considered responsible for 46% of the infection-associated cancers in the economically developed and 29% in less affluent settings (de Martel *et al.*, 2012).

In developing countries, *H. pylori* infection is acquired predominantly during childhood, whereas in the more developed regions, the infection is less common in children, and gradually increases with age; the highest incidence rates are observed in childhood or in adolescence (Kivi and Tindberg, 2006). Understanding the early-life determinants of *H. pylori* infection may enable preventive strategies to further decrease the frequency of infection.

Transmission has been described to occur primarily between mothers and their offspring (Kivi and Tindberg, 2006) and among siblings (Kivi and Tindberg, 2006; Weyermann *et al.*, 2006); person-to-person contact is the most commonly implicated mechanism, through fecal/oral, oral/oral, or gastric/oral pathways (Brown, 2000). Childcare attendance promotes interpersonal contacts, and is a well-accepted risk factor for acute respiratory infections and diarrhea (Barros, 1999; Ochoa Sangrador *et al.*, 2007). It may also contribute towards an increased risk of *H. pylori* infection to different extents depending on the number and the characteristics of the children being cared for together (e.g. age; parental socioeconomic status), duration of exposure, and hygiene practices in the childcare setting (Ochoa Sangrador *et al.*, 2007).

Therefore, we aimed to quantify the association between childcare attendance and *H. pylori* infection through a systematic review and meta-analysis.

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Materials and methods

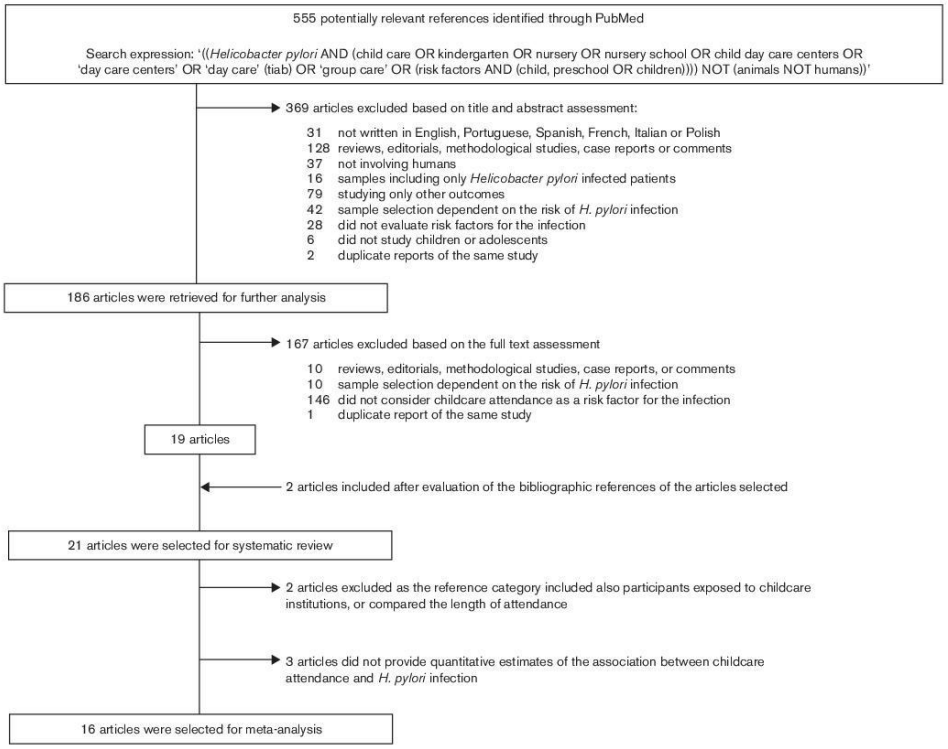
Literature search

PubMed was searched from inception to July 2012 to identify studies addressing the association between childcare attendance and *H. pylori* infection in childhood or adolescence. The search expression is presented in the systematic review flowchart (Fig. 1).

Two reviewers (J.B. and H.C.) independently evaluated the original studies in three consecutive steps following predefined criteria. In the first step, the eligibility was determined on the basis of the information presented in the title and in the abstract. When the abstract was not available, the study was considered for evaluation in step 2, except when the title provided sufficient information to unequivocally exclude it. The full texts of the studies selected for step 2 were assessed to determine the eligibility and availability of relevant data; in step 3, they were re-evaluated to determine their eligibility for meta-analysis.

Studies were excluded according to criteria defined *a priori*: (a) not written in English, Portuguese, Spanish, French, Italian, or Polish; (b) reviews, comments, or editorials; (c) studies not involving humans (e.g. assessment of antibiotic resistance); (d) studies evaluating samples that included only *H. pylori*-infected patients; (e) studies addressing the association between *H. pylori* and other health outcomes (e.g. respiratory diseases, blood parameters); (f) studies involving samples of individuals not representing the general population (e.g. children undergoing endoscopy for diagnostic procedures), or involving only children attending a childcare center; (g) reports not providing data on the relation between *H. pylori* infection and childcare center attendance; (h) studies evaluating the exposure to childcare

Fig. 1



Systematic review flowchart.

attendance in childhood and assessing the *H. pylori* infection in adulthood; and (i) repeated reports of the same study.

The decisions taken by the two researchers were compared in all steps and the discrepancies were discussed until consensus or resolved involving a third researcher (N.L.).

The literature search was further complemented by backward citation tracking among the articles considered eligible for the systematic review.

Data extraction

Two reviewers (J.B. and H.C.) independently abstracted information on the following variables: (a) year of publication; (b) country and region; (c) study design; (d) sample characteristics (sample size and age distribution); (e) diagnostic methods used to assess *H. pylori* infection status; (f) childcare attendance (type of setting and duration of exposure); and (g) adjusted measures of association, mostly odds ratio (OR), whenever available in the original study, or crude OR, or the necessary information to calculate them, along with the corresponding precision estimates.

Age-specific estimates were extracted whenever available, as well as estimates referring to different periods of duration of childcare attendance.

The discrepancies in the data extracted by the two reviewers were discussed and resolved by consensus or by involving a third researcher (N.L.) whenever necessary.

Meta-analysis

In the studies presenting more than one measure of childcare attendance, either regarding the type of setting, duration of exposure, or number of children cared for together, we considered the data corresponding to the longest exposure, the largest group of children, or the largest number of children exposed, as applicable. When a study provided crude and adjusted ORs, the adjusted estimates were selected for meta-analysis.

The DerSimonian and Laird method was used to compute summary estimates of the association between childcare attendance and *H. pylori* infection, and respective 95% confidence intervals (CIs). Heterogeneity was quantified using the I^2 statistic (Higgins and Thompson, 2002). Stratified analyses and metaregression were used to explain the heterogeneity of the results across studies with different methodological characteristics and according to the prevalence of *H. pylori* infection in the adult population of the countries where the studies were carried out, as described in a previous review (Lunet and Barros, 2003). The latter was used as a surrogate of the risk of infection in the general population of the countries, and in particular its incidence in the younger age groups. We considered the countries Germany, Greenland, Ireland, Italy, Sweden, and the USA (prevalences ranging between 30 and 45%) as having a low prevalence, corresponding to a low risk of infection during childhood, and Brazil, Japan, Poland, Portugal, South Africa, Russia, and Israel (prevalences ranging between 66 and 88%) as having a high prevalence of infection, and therefore a higher risk of infection in early life.

The statistical analysis was carried out using STATA, version 9.2 (Stata Corp., College Station, Texas, USA).

Results

Systematic review

We identified 21 studies eligible for the systematic review, involving samples recruited in 17 countries. Their main characteristics are presented in Appendix A1, Tables A1 and A2.

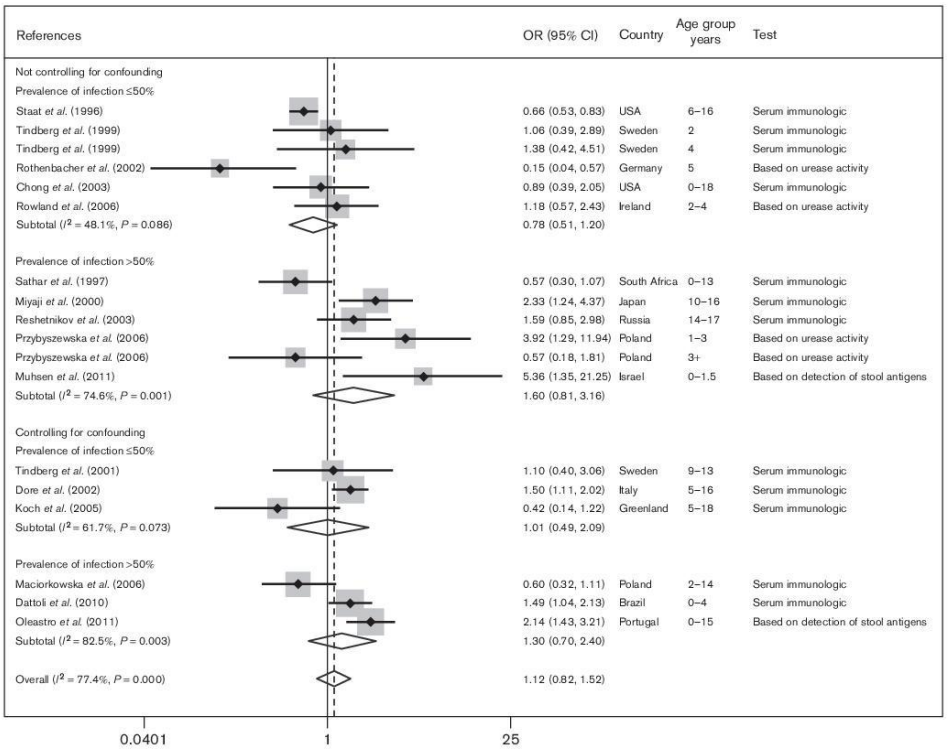
The association between childcare attendance and *H. pylori* infection was assessed in samples of participants with ages ranging from 0 to 17 years; in five studies, the sample included only participants above the age of elementary school enrollment. All studies involved noninvasive or minimally invasive tests for the diagnosis of infection, mainly using samples of blood, expired air, and stool. The characteristics of group care, such as the number of participants attending and age groups involved, or duration of exposure were seldom addressed (Koch *et al.*, 2005; Muhsen *et al.*, 2011). Only six studies provided adjusted estimates of the association between childcare attendance and *H. pylori* infection, and none presented this as a primary objective.

Sixteen studies were considered for meta-analysis (Staat *et al.*, 1996; Sathar *et al.*, 1997; Tindberg *et al.*, 1999; Miyaji *et al.*, 2000; Tindberg *et al.*, 2001; Dore *et al.*, 2002; Rothenbacher *et al.*, 2002; Chong *et al.*, 2003; Reshetnikov *et al.*, 2003; Koch *et al.*, 2005; Maciorkowska *et al.*, 2006; Przybyszewska *et al.*, 2006; Rowland *et al.*, 2006; Dattoli *et al.*, 2010; Muhsen *et al.*, 2011; Oleastro *et al.*, 2011). Among the others, two reported no association between childcare attendance and *H. pylori*, but did not provide quantitative estimates or information on the direction of the association, or enough information to compute it (Malaty *et al.*, 1996; Daugule *et al.*, 2001). In the remaining three (Kurosawa *et al.*, 2000; Jelavic *et al.*, 2007; Sýkora *et al.*, 2009), the reference category also included participants who attended childcare or compared the different duration of exposure without providing information to compute a measure of association, although tended to show a higher risk among those exposed for a longer period.

Meta-analysis

The summary OR was 1.12 (95% CI: 0.82–1.52, $I^2 = 77.4\%$), similar for studies providing only crude estimates (OR = 1.10, 95% CI: 0.72–1.68) and for those adjusted for potential confounders (OR = 1.22, 95%

Fig. 2



Meta-analyses of studies evaluating the association between childcare attendance and *Helicobacter pylori* infection. CI, confidence interval; OR, odds ratio.

CI: 0.83–1.78). The association was stronger for populations with high prevalence of infection, both when considering crude (OR = 1.60, 95% CI: 0.81–3.16) and adjusted estimates (OR = 1.30, 95% CI: 0.70–2.40) (Fig. 2). When the infection was evaluated in children aged 3 years or younger, the combined estimates were higher (OR = 2.00, 95% CI: 0.94–4.29, $I^2 = 55.0\%$) than those for studies that only evaluated children aged older than 3 years of age (OR = 0.99, 95% CI: 0.71–1.38, $I^2 = 79.9\%$).

Studies relying on serum immunologic tests or tests based on urease activity yielded lower OR estimates (OR = 1.03, 95% CI: 0.75–1.42, $I^2 = 74.3\%$ and OR = 0.84, 95% CI: 0.26–2.68, $I^2 = 79.6\%$, respectively) than those based on the detection of stool antigens (OR = 2.65, 95% CI: 1.24–5.66, $I^2 = 36.4\%$).

In multivariate metaregression, there was no significant association with any of the variables described above; taking them into account contributed to a reduction of I^2 to 67%.

Discussion

The available evidence does not indicate a strong association between childcare attendance and *H. pylori* infection, and this appears to be an increased risk only in the settings where the overall frequency of infection is high. None of the original reports presented the relation between childcare attendance and *H. pylori* infection as a primary objective, which may have contributed towards the large heterogeneity observed.

We carried out stratified and metaregression analyses in an attempt to identify more homogeneous subgroups

of studies, but the methodological characteristics available allowed minor improvements in the I^2 statistic. This is not surprising, as only crude OR estimates could be obtained from more than half the studies and the effect of uncontrolled confounding on the individual OR estimates is likely to differ considerably across studies, according to the setting-specific social determinants of exposure to childcare; this is reflected in the large interstudy variation in the proportion of children attending childcare centers, which ranged between 10% in Japan in 2000 (Miyaji *et al.*, 2000) and 97% in Germany in 2002 (Rothenbacher *et al.*, 2002). However, the pooled ORs were similar for studies providing multivariate or crude ORs.

There were also important differences across studies in the age distribution of the participants. Although a few investigations included children within a relatively narrow age range younger than the age of 6, most investigations also evaluated adolescents. This is relevant for the interpretation of the findings and to understand heterogeneity. On the one hand, the effect of childcare may vary with age, as observed for other infections (Barros, 1999). For example, in the studies carried out by Tindberg *et al.* (1999) and Przybyszewska *et al.* (2006), the OR estimates were different across the age groups considered. On the other, the larger the lag between the exposure to childcare and the assessment of infection status, the more likely it is that the OR estimates reflect the effect of factors other than childcare, and the strength of the associations is attenuated because the rates of infection may remain high throughout adolescence (Lunet and Barros, 2006). Therefore, the lack of results stratified by narrow age groups precludes a proper assessment of the relation between childcare and infection, as well as the explanation of heterogeneity.

Another limitation of several studies included in this review is the use of measures of exposure to childcare that seldom account for the characteristics of the childcare setting or duration of exposure. The results of the study carried out by Sýkora *et al.* (2009), not included in the meta-analysis because no OR estimates were provided or computable with the available data, yielded different conclusions when finer measures of exposure were considered; although there was no association when the time a child started to attend a daycare center was considered, the total length of attendance was longer in infected compared with noninfected children. Another example that shows the importance of knowing the care trajectory of the children since their enrollment is provided by the study carried out by Rothenbacher *et al.* (2002). The authors presented results for the relation between attendance at a daycare center (only 6% of the children were exposed) and for attendance at kindergarten (97% were exposed); the latter yielded a negative association (OR = 0.15) and the former yielded a positive association (OR = 3.56). These differences probably reflect a different distribution of the confounders, but are also likely to be explained by the fact that these different types of childcare include children with different ages.

The stronger associations between childcare attendance and *H. pylori* infection observed in countries where the prevalence of infection was high may reflect an increased number of interactions with infected children, making child-to-child interactions more likely to result in new infections.

The conclusions reached by systematic reviews and meta-analyses depend on the comprehensiveness of the search strategy and on the criteria for study inclusion and selection of data for quantitative synthesis, in addition to the quality of the evidence being reviewed. Although the heterogeneity of results across studies precludes a sound assessment of 'small studies effects' through the analysis of a funnel plot or an analogous method, the search strategy used for the review makes publication bias unlikely. On the one hand, the review was based on a comprehensive search expression that referred to risk factors for infection in general, in addition to including terms that identify specifically different forms of care of children, which contributes toward the high sensitivity of the database search. On the other, the assessment of the relation between childcare and infection was not a primary objective of these studies, and therefore, the nature of these findings is unlikely to have resulted in publication bias.

Conclusion

The potential effect of childcare as a risk factor for *H. pylori* infection is biologically plausible, especially in settings where the prevalence of infection is expectedly high during childhood. However, most studies did not address the effect of the characteristics of the childcare institutions or the duration or intensity of exposure, leaving considerable scope for improvement of our understanding of the contribution of this modifiable exposure in the occurrence of *H. pylori* infection.

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Conflicts of interest

There are no conflicts of interest.

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Appendix A1.

Table A1 and Table A2

Table A1 Characteristics of the studies identified by systematic review and excluded from the meta-analysis because they did not fulfill the eligibility criteria

References	Country, region	Participants' description[a]	Age group (years)	Sample	Method to assess infection status	Type of childcare	Description of the main findings	Reason for exclusion
Sýkora *et al.* (2009)	Czech Republic, Pilsen, and the surrounding rural area	'The general pediatric population (...) was chosen as the study population. (...) On a foundation of a prospective cross-sectional population-based survey, this study focused on baseline epidemiological data and risk factors for *H. pylori* infection in the asymptomatic pediatric population'	0–15	Stool	ELISA[b]	Nursery or kindergarten	'There were no differences in the time when a child started to attend daycare center (nursery, kindergarten) when we compare groups of *Helicobacter pylori*-positive and -negative status (3.10±1.31 vs. 3.10±1.25; $P>0.05$). The total length of attendance to daycare centers and school was significantly longer in infected compared with noninfected children (7.36±3.64 vs. 5.71±3.59; $P<0.001$)'	The exposure considered was duration of attendance of daycare center and school at the present
Jelavic *et al.* (2007)	Bosnia and Herzegovina, Mostar	'Children (…) who attended the Department of Otorhino-laryngology (…) for tonsillectomy'	4–14	Blood	Immuno-chromato-graphic[b]	Kindergarten or school with more than 20 children	'The data about seronegative group and seropositive group are shown in the table. There was no significant difference between these groups comparing (…) attending a children's collective (χ^2, 1.3; *d.f.*=2; $P=0.5$)'	The exposure considered was attending at a kindergarten/school with more than 20 children
Daugule *et al.* (2001)	Latvia, Riga	'Consecutive children (…) without symptoms from the gastrointestinal tract, who visited their doctor for a general checkup or because of minor health problems'	1–12	Expired air	Urea breath test	Daycare center	'The univariate associations of some of the studied risk factors with *H. pylori* positivity are shown in the table. (…) The other possible risk factors did not demonstrate a significant association with *H. pylori* infection'	OR or suitable information to compute it was not provided
Kurosawa *et al.* (2000)	Japan, Nagano	'325 students in the first grade of three elementary schools and on 307 students in the second grade of a junior high school'	6 and 14	Saliva	ELISA[c]	Nursery school or kindergarten	'Factors related to saliva *H. pylori* positivity were months in a nursery school or kindergarten OR =4.0; 95% CI 1.8–9.1 (0–36 months in nursery school vs. 36+ months)'	The reference class considered patients who had not been exposed and exposed for a period lower than 36 months
Malaty *et al.* (1996)	South Korea, Seoul	'Adults and children who visited a health screening center (…) for routine health examination'	1–19	Blood	ELISA[c]	Daycare center	'The questionnaire for children was designed to obtain information regarding (…) whether the child attended a daycare center and until what age (…). Risk factors that were significant in the univariate analysis were used in the multiple logistic regression model (daycare attendance was not included)'	OR or suitable information to compute it was not provided

CI, confidence interval; OR, odds ratio.
[a]All studies had a cross-sectional design.
[b]*Helicobacter pylori* antigens evaluated.
[c]IgG antibodies evaluated.

Table A2 Characteristics of the studies included in the meta-analysis

References	Country, region	Participants description[a]	Age group (years)	Sample	Method to assess infection status	Type of childcare	Category of exposure used for OR estimation	Reference category used for OR estimation	OR (95% CI)	Confounding control
Oleastro *et al.* (2011)	Portugal, Lisboa	'Children were recruited at the moment of attendance for the vaccination program'	0–15	Stool	Immuno-enzymatic[e]	Nursery or kindergarten	Attendance at nursery or kindergarten	Looked after at home	2.14 (1.43–3.21)	Adjusted for: Age Sex
Muhsen *et al.* (2011)	Israel, north region	'Mothers of eligible infants (…) were recruited through the local family health clinics'	0–1.5	Stool	Enzyme immune assay[e]	Daycare center	Attendance at large daycare center (>5 children)	No attendance	5.36 (1.30–20.44)	No
							Attendance at the daycare center for ≤6 h/day	No attendance	2.12 (0.69–6.06)[b]	No
							Attendance at the daycare center for >6 h/day	No attendance	7.14 (1.41–34.64)[b]	No
Dattoli *et al.* (2010)	Brazil, Salvador	'Three baseline surveys were carried out (…) allowing different children (…) to be recruited and followed-up. These three surveys were part of a study aimed at evaluating the impact of a sanitation programme on the incidence of childhood diarrhea (…). In 2005, 1445 of these children were resurveyed'	ND (mean age: 6.8)	Blood	ELISA[f]	Nursery	Attendance at nursery	No attendance	1.49 (1.04–2.12)	Adjusted for: Age Sex Number of siblings Paved road (1997–2003 and 2005) Flush toilet
Przybyszewska *et al.* (2006)	Poland, Cracow	'Randomly selected healthy children (…) attending Healthy Child Centers in Cracovia for their immunization or physical assessment of their health'	1–3 and 3–4	Expired air	Urea breath test	Crèches or kindergartens	Attendance at a crèche or a kindergarten	No attendance	1–3 years: 3.92 (1.29–11.94)[h] 3–4 years: 0.57 (0.18–1.81)[h]	No
Maciorkowska *et al.* (2006)	Poland, North-East	'Randomly selected from a National Health Ministry program to study the infection by *Helicobacter pylori* infection'	2–14	Blood	ELISA[f]	Nurseries or kindergartens	Attendance at a nursery or a kindergarten	Looked after at home	0.60 (0.32–1.11)[h]	No
Rowland *et al.* (2006)	Ireland, Dublin, Mallow, and Kingscourt	'The aim of this study was to prospectively follow-up a group of healthy children (…). Nineteen family doctors were approached to provide patients for the study'	2–4	Expired air	Urea breath test	Crèches or preschool	Attendance at crèche or preschool	No attendance	1.18 (0.57–2.43)	No
Koch *et al.* (2005)	Greenland, Sisimiut	'Blood samples from preschool children were obtained from participants in a population-based cohort study'	5–6	Blood	ELISA[f]	Childcare center	No attendance	Attendance at a childcare center before 6 years of age	0.42 (0.14–1.22)	Adjusted for: Age Sex
							Attendance at a daycare center for <2 years before 6 years of age	Attendance at childcare center 5–6 years before 6 years of age	0.45 (0.11–1.86)[b]	Adjusted for: Age Sex
							Attendance at a daycare center for 2–4 years before 6 years of age	Attendance at childcare center 5–6 years before 6 years of age	0.7 (0.29–1.71)[b]	Adjusted for: Age Sex

Chong *et al.* (2003)[c]	USA, Indianapolis, Brooklyn, Columbus, Detroit, Greenville, Huntington, Atlanta, New Orleans, Amarillo, Houston, San Francisco, and Gainesville	'922 children were recruited from 12 children's hospitals or medical centers'	≤ 6	Blood	Enzyme immune assay[f]	Daycare center	No attendance	Attendance to a childcare center	Prevalence ratio = 0.89 (0.39–2.06)	No
Reshetnikov *et al.* (2003)	Russia, Siberia (Novosibirsk)	'Four secondary schools were selected randomly for the study. All the students in forms 9–11, aged 14–17 years, were invited to participate'	14–17	Blood	Enzyme immune assay[f]	Nursery school	No attendance	Attendance at nursery school	1.59 (0.85–2.98)	No
Rothenbacher *et al.* (2002)	Germany, Ulm	'A sample of preschool children (…) who were screened for school fitness'	5–7	Expired air	Urea breath test	Daycare center	No attendance	Attendance at daycare center	3.56 (1.19–10.70)[b,h]	No
							No attendance	Attendance at kindergarten	0.15 (0.04–0.57)[h]	No
Dore *et al.* (2002)	Italy, Sardinia (Porto Torres)	'All elementary and middle schools located in the villages and in the urban area were included'	5–16	Blood	ELISA[f]	Daycare center	No attendance	Attendance at a daycare center	1.5 (1.1–2.0)	Adjusted for: Age Sex Occupation of the head of the household Contact with dogs and other animals Breast feeding
Tindberg *et al.* (2001)	Sweden, Stockholm	'All 11 invited schools accepted participation'	9–13	Blood	ELISA[f]	Daycare center (15–20 children per group) or some other form of day care	Looked after at home	Attendance at a childcare center	1.1 (0.4–3.1)[d]	Adjusted for: Sex Household SES Family size Family origin
Miyaji *et al.* (2000)	Japan, not further specified	'Serum was collected from 1684 individuals (…) who had received public health examinations'	10–16	Blood	ELISA[f]	Nursery school	No attendance	Attendance at nursery school for at least 1 year	2.33 (1.24–4.36)	No
Tindberg *et al.* (1999)	Sweden, Stockholm	'Within the framework of a pertussis vaccine trial, serial capillary blood samples were drawn from each child at the age of 6 mo, 8 mo, 10 mo, 18 mo, 2 y and 4 y of age'	2 and 4	Blood	ELISA[g]	Daycare center	Looked after at home at 2 and 4 years old	Attendance at a childcare center at 2 and 4 years old	2 years: 1.06 (0.39–2.89)[h, i] 4 years: 1.38 (0.42–4.51)[h, i]	No
Sathar *et al.* (1997)	South Africa, Umlazi	'School children (…) from 10 schools in Umlazi were selected randomly'	0–13	Blood	ELISA[f]	Crèche	No attendance	Attendance at crèche	0.57 (0.30–1.07)[h]	No
Staat *et al.* (1996)	USA	'The National Health and Nutrition Examination Survey (NHANES)'	6–16	Blood	ELISA[f]	Daycare center	No attendance	Attendance to daycare center	0.66 (0.53–0.83)[h]	No

CI, confidence interval; ND, not defined; OR, odds ratio; SES, socioeconomic status.
[a]All studies had a cross-sectional design except Muhsen *et al.* (2011), Oleastro *et al.* (2011), Dattoli *et al.* (2010), Rowland *et al.* (2006) and Tindberg *et al.* (1999), although in these studies the relation between childcare attendance and *H. pylori* infection was quantified as in a case-control or cross-sectional study.
[b]Estimates not considered for meta-analysis.
[c]Only the crude estimates were considered, as the adjusted ones also included participants who were referred because of gastrointestinal complains.
[d]Estimate considering only children born in low-prevalence areas.
[e]*Helicobacter pylori* antigens evaluated.
[f]IgG antibodies evaluated.
[g]IgG and IgA antibodies evaluated.
[h]OR estimates and corresponding 95% confidence intervals computed by the authors of the present review using Epi Info 7.
[i]Both estimates were included in meta-analysis.

GENERAL DISCUSSION

This thesis aimed to identify factors associated with *H. pylori* infection at different ages (distinguishing the potentially critical periods of childhood and adolescence, in addition to adulthood). To accomplish its specific objectives, we conducted three studies based in different Portuguese cohorts and a systematic review and meta-analysis.

In our work we were able to depict the overall age-distribution of *H. pylori* infection in this Portuguese area (figure 5): a high prevalence since young ages and increasing with age (studies I, II and III). Comparing to previous studies, it seems that the prevalence is not decreasing in more recent cohorts (figure 6).

Figure 5: Age-distribution of prevalence of *H. pylori* in Porto

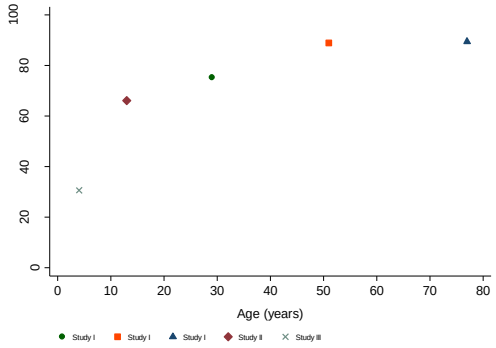
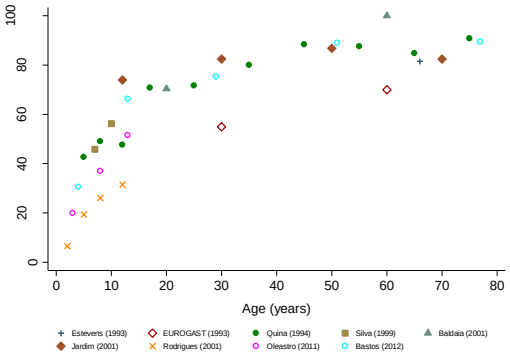


Figure 6: Age-distribution of prevalence of *H. pylori* in Portugal*



* This figure is an adaptation of a previous picture from Lunet N. (87).

Due to the relation between *H. pylori* infection and gastric cancer (28, 29, 88), the results from study I, II and III strongly suggest that gastric cancer incidence and mortality in this Portuguese setting will remain among one of the highest in the world, especially among less educated individuals.

In adults, we were able to identify two distinct scenarios. Prevalence increases across age groups in the more educated subjects and decreases with education in the younger. Moreover, living in a more deprived neighbourhood was associated with a higher risk of infection, but only among the younger and more educated participants (study I).

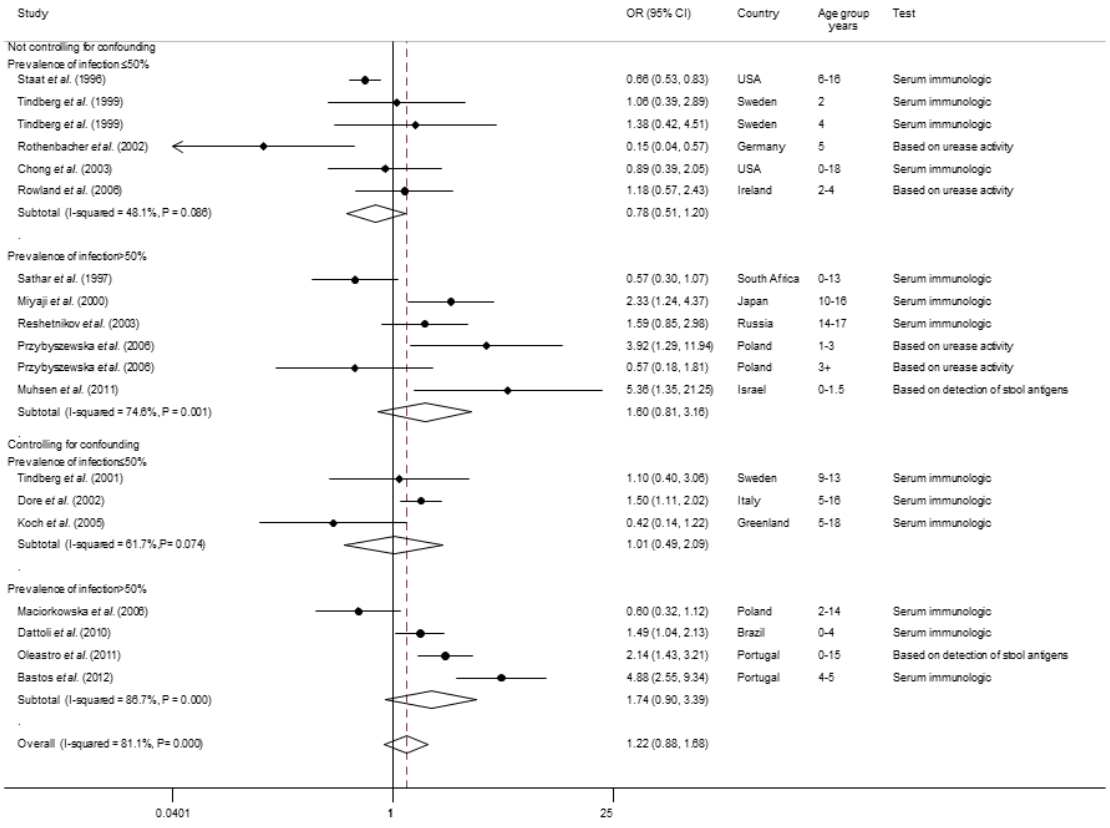
H. pylori infection is the major risk factor for gastric cancer and the possibility of it being considered a necessary cause for the occurrence of this outcome is currently under discussion (89). If this hypothesis is confirmed, the interventions for primary prevention of the infection may be sufficient to prevent gastric cancer. Additionally,

some of the gastric precancerous lesions that are relatively frequent in populations with a high frequency of infection may be irreversible (90, 91). Therefore, the strategies for prevention of the infection in early life are more likely to yield an effective control of gastric cancer.

In adolescence, our results show a high prevalence of infection at the age of 13, but also a high incidence between the ages of 13 and 17, confirming that the acquisition in this period is not negligible and contributes to the high prevalence later in life. Additionally, social class, represented by parental education, is a relevant determinant of the prevalence at baseline, and smoking is a modifiable exposure associated with the incidence of infection during adolescence (study II).

In young children (study III), we found that the education of the mother, probably measuring socio-economic status, is associated with the acquisition of the infection. We also identify the attendance of a day-care centre as a risk factor for infection and that infection increases significantly with the cumulative time of attendance of day-care centres or homes since birth. These findings are compatible with the importance of close contacts in the acquisition of *H. pylori* infection and the faecal-oral and oral-oral ways of transmission. These results are supported by the results from our systematic review (study IV), where we were able to identify a relation between child-care attendance and the acquisition of *H. pylori* infection, reinforcing the role of child-care attendance in the early acquisition of *H. pylori* infection, especially in settings with high prevalence of infection, as is Portugal (figure 7).

Figure 7: Meta-analyses of studies evaluating the association between child-care attendance and *Helicobacter pylori* infection.



Socio-economic status is a known recognized risk factor for the acquisition of *H. pylori* infection (15, 59, 66) and we confirm it with our results (study I, II and III). However, it is not a modifiable exposure, or at least it is very difficult to change in relatively small period of time. The results from study III identify day-care centres and day-care homes as targets for interventions aiming the prevention of the infection during childhood. The findings from study II identify smoking as a risk factor, showing that there is potential for intervention in the reduction of *H. pylori* infection during adolescence and this should be taken into account when estimating the potential benefits of tobacco control measures at early ages.

The main conclusions of this work are:

- In adults, the prevalence of *H. pylori* infection in Portugal remains among the highest in Europe, particularly among less educated individuals; taking this into account, stomach cancer incidence and mortality rates in Portugal are likely to remain among the highest in the World during the next decades.
- *H. pylori* infection is a frequent and early event in Portugal. We identified smoking as a modifiable risk factor for infection during adolescence, and showed that the risk of infection in early childhood is increased by day-care attendance; these are potential targets for prevention of *H. pylori* infection in early life.

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